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L5	(single adj chain) same (class adj II)	33	L5
L4	(single adj chain) near (class adj II)	8	L4
L3	L2 and MHC	41	L3
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US-PAT-NO: 6309645
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TITLE: MHC molecules and uses thereof

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INVENTOR-INFORMATION:

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US-CL-CURRENT: 424/192.1; 424/185.1, 424/198.1, 435/69.7, 530/350, 530/387.1, 530/395

CLAIMS:

What is claimed is:

1. A method of suppressing an immune response in a mammal comprising administering to the mammal an effective amount of a single-chain class II MHC molecule comprising loaded or covalently linked presenting peptide and that comprises covalently linked in sequence: 1) a class II .beta. chain, 2) a single chain linker sequence, and 3) a class II .alpha. chain, wherein the molecule does not include an MHC class I chain and the chain of 1) or 3) or both 1) and 3) lack a functional transmembrane domain.

2. The method of claim 1 wherein the single-chain class II MHC molecule is soluble.

3. The method of claim 1 wherein the single-chain class II MHC molecule comprises covalently linked in sequence: 1) presenting peptide, 2) class II .beta. chain, 3) the single chain linker, and 4) the class II .alpha. chain.

4. The method of claim 1 wherein the single chain linker is covalently linked to the N-terminus of the .alpha. chain.

5. The method of claim 1 wherein the single chain linker is covalently linked between the carboxyl terminus of a .beta.2 domain of the .beta. chain and an .alpha.1 domain of the .alpha. chain.

6. The method of claim 1 wherein the mammal suffers from an autoimmune disorder.

7. The method of claim 1 wherein the mammal suffers from a multiple sclerosis, insulin-dependent diabetes mellitus or rheumatoid arthritis.

8. The method of claim 1 wherein the mammal is undergoing transplant surgery.

9. The method of claim 1 wherein the single-chain class II MHC molecule is linked to an immunoglobulin.

10. The method of claim 1 wherein the single-chain class II MHC molecule is fused to constant regions of an immunoglobulin molecule.

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Refolding and Reconstitution of Functionally Active Complexes of Human Leukocyte Antigen DR2 and Myelin Basic Protein Peptide from Recombinant α and β Polypeptide Chains*

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Major histocompatibility complex (MHC) class II molecules are cell surface heterodimeric glycoproteins consisting of one α and one β polypeptide chain of similar size. These molecules play a critical role in immune recognition by displaying processed antigens to CD4-positive T helper cells. Several attempts to express the MHC class II molecules by recombinant methods in various systems resulted in either failure or poor recovery of the intact heterodimer. The present study describes our successful effort to refold and reconstitute HLA DR2 heterodimer from individually expressed α and β polypeptide chains lacking the transmembrane hydrophobic regions in *Escherichia coli*, in the presence of an immunodominant epitope analog from human myelin basic protein (b-MBP(83–102)Y^{83}). The reconstituted DR2 heterodimer complex was selectively purified from unfolded α and β chains using heterodimer-specific monoclonal antibody (L243) coupled to a solid support. The detection of two polypeptide chains in the purified refolded DR2-peptide complex preparations was accomplished by Western blot analysis and enzyme-linked immunosorbent assay using heterodimer- and chain-specific polyclonal antibodies, and the presence of equimolar amounts of both α chain and β chain in the reconstituted complex preparation was confirmed by a double label experiment. The quantitation of the bound peptide in complex preparation was measured by incubating two chains in the presence of ^{125}I-labeled peptide. An increase in the yield of refolded and reconstituted DR2-peptide complexes was observed with increasing peptide concentration in the reaction mixture. Finally, the functional activity of the reconstituted DR2 complexes was measured by their ability to stimulate γ-interferon production by SS8T cloned T cells in an antigen-specific and dose-dependent manner. These results demonstrate that biologically active complexes of human DR2·b-MBP(83–102)Y^{83} can be prepared by proper folding of human leukocyte antigen DR2 α and β chains in the presence of antigenic peptide. The yield of such DR2 heterodimers with bound peptide is several thousand-fold higher over native DR2 purified from transformed B cells. Since purified MHC class II-peptide complexes have been shown to prevent autoimmune diseases in various animal models, reconstituted heterodimer complexes may have significant clinical relevance in antigen-specific treatment of various autoimmune diseases. In addition, such complexes with increased yield will provide better understanding of the trimolecular interactions between MHC-peptide and T cell receptor.

The presentation of antigens to CD4-positive T helper cells involves the binding of processed antigenic peptides to MHC[1] class II molecules on the surface of antigen-presenting cells (1–5). Purified MHC class II molecules isolated from cell surfaces are also known to bind antigenic peptides *in vitro* (6–8). The yields of purified MHC class II molecules from various cell sources are usually very poor and represent less than 0.5% of the total protein pool (9–11). The low yield of purified MHC class II molecules has always been an important limiting step in elucidating structure-function correlation studies of these molecules. Various attempts to clone and express MHC class II molecules have been reported recently in prokaryotic (12) and eukaryotic systems (13–16). The recombinant heterodimers prepared by these methods also represent very low yields due to difficulties in refolding.

Structural information of MHC class II-peptide interaction has become available recently from x-ray crystallographic studies (17, 18). The peptide binding groove of MHC class II molecules consists of the α1 and β1 domains with eight-stranded β-pleated sheets as the floor of the peptide binding groove with extended α helices. Peptides play a related and distinct role in the structure and conformational maturation of MHC class II molecules (19). Approximately 2000 different peptides may bind to MHC class II molecules (20). Therefore it is difficult to obtain a peptide-free preparation of MHC heterodimeric molecules because of the slow dissociation rate constant of the bound peptides (13). To overcome this problem some of the MHC class II molecules are expressed in eukaryotic systems which lack the peptide processing machinery (13–16).

The folding of MHC class II heterodimer also appears to be an inherently difficult problem. Unlike MHC class I molecules, where the α1 and α2 domains from the same polypeptide form an intramolecular dimer (21, 22), the peptide binding site of class II molecules consists of two separate domains, α1 and β1, from two individual polypeptides (17). The MHC class II protein, therefore, differs from other heterodimeric proteins of the immune system that have been successfully folded *in vitro*, where each domain is composed of protein segments of a single polypeptide chain (23–25). Reconstitution of functionally active murine MHC class II peptide complexes from *Escherichia coli*-

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[1] The abbreviations used are: MHC, major histocompatibility complex(es); MBP, myelin basic protein; HLA, human leukocyte antigen; TCR, T cell receptor; IFN, interferon; TMB, 3,3′,5,5′-tetramethylbenzidine; (−TM), chains lacking transmembrane and cytoplasmically exposed regions; PBS, phosphate-buffered saline; HRP, horseradish peroxidase; PAGE, polyacrylamide gel electrophoresis; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction.

expressed, unpurified individual α and β chains have been demonstrated recently (12). The yield of such active heterodimer represents 0.5–2% of the starting protein. In this report, we describe the refolding of *E. coli*-expressed recombinant human α and β chains lacking the transmembrane regions followed by reconstitution of biologically active HLA DR2. MBP peptide complexes with a yield of over 20% of the starting protein concentration.

MATERIALS AND METHODS

Cell Lines, Antibodies, and Chemicals—The hybridoma cell line L243, producing monoclonal antibodies against monomorphic human HLA DR molecules, was obtained from American Type Culture Collection, Bethesda, MD. Homozygous lymphoblastoid cell lines, GM 03107 expressing HLA DR2 and GM 08067 expressing HLA DR3, were obtained from the National Institute of General Medical Sciences (NIGMS) human genetic mutant cell repository (Corinell Institute of Medical Research, NJ). Polyclonal antibodies against individual α and β chains were raised in rabbits using recombinant purified α and β chains lacking the transmembrane region as antigens. Both anti-α and anti-β polyclonal antibodies were purified by affinity chromatography where purified antigens were coupled to activated Sepharose 4B column. Anti-human γ-IFN monoclonal antibody and rabbit anti-human γ-IFN polyclonal antibody were obtained from Endogen. HRP-conjugated rabbit IgG was purchased from Jackson Immunoresearch Laboratories. Human γ-IFN was obtained from Boehringer Mannheim. The color developing substrate 3,3',5,5'-tetramethyl benzidine (TMB) was obtained from Moss, Inc.

Cloning and Expression of DR2Dw2 (−TM) Single Chains—The vector for expression of single chain MHC was derived from the pET system of plasmids (26). The vector pET-11a (Novagen) was used to construct an expression vector p27313 by modifications of cloning sites. DR2 α and DRB5*0101 chains lacking the transmembrane regions were PCR-amplified using poly(A)$^+$ mRNA from GM 03107 lymphoblastoid cells. The PCR primers were synthesized based on the sequences obtained from the GeneBank™ data base for human DR2Dw2 alleles. The top strand primer for both chains included a 5- amino acid sequence of the ϕ-10 gene of bacteriophage T7 followed by a translational stop codon and the initiator methionine for the chain of interest (27). The amplified sequences were cloned into p27313 using *Bam*HI and *Eco*RI restriction enzymes whose sites were tailored in the PCR primers. Insert-containing clones were identified and sequenced. The tetracycline resistance gene was cloned into the plasmids containing DR2 α and β chains in order to facilitate scale-up culturing. The resulting plasmids - p329129 and p33425 expressing DR2 α-TM and DRB5*0101-TM chains were transformed into the *E. coli* expression host W3310/DE3. Induction cultures were grown at 37 °C in L-broth containing 0.4% glucose, 100 μg/ml ampicillin, and 15 μg/ml tetracycline. Cells were induced in mid-log growth by addition of isopropyl-β-D-thiogalactopyranoside to a final concentration of 0.4 mM and allowed to grow for an additional 2 h. Cells were harvested and used for inclusion body preparation.

Purification of DR2 α and β Chains Lacking the Transmembrane Region—The detailed procedure for the purification of α and β chains lacking the transmembrane region from *E. coli* inclusion body preparations has been described recently (28). Briefly, the α chain *E. coli* inclusion bodies were solubilized in 25 mM phosphate buffer, pH 7.4, containing 8 M urea and 20 mM dithiothreitol and purified by ion exchange chromatography using High Q-50 resin (Bio-Rad). The recombinant β chain was purified by one-step gel filtration chromatography using Sephacryl S-100 resin packed in a Pharmacia XK50 (2.5-cm diameter × 100-cm height) column. Both α and β chain fractions were collected and analyzed by SDS-PAGE electrophoresis using a LabLogix silver staining kit (Belmont, CA). Individually pooled α and β chains showed purity levels greater than 95% with recovery of 52 and 86%, respectively.

Purification of Human HLA DR2 and DR3 from Lymphoblastoid Cells—Purification of HLA DR2 from Ebstein-Barr virus-transformed lymphoblastoid cells was carried out as described earlier (29) with some minor modifications. Triton X-100 cell lysate was applied onto L243-coupled Sepharose-4B column, and the bound DR2 was eluted in phosphate buffer containing 0.05% *n*-dodecyl-β-D-maltoside detergent at pH 11.3. Fractions were immediately neutralized with 1 M acetic acid, and the DR2 pool was collected through a DEAE ion exchange column in a phosphate buffer containing 0.5 M NaCl and 0.05% *n*-dodecyl-β-D-maltoside, pH 6.0. Purified protein was then filtered through a 180-kDa membrane, dialyzed against PBS for 24 h at 4 °C and characterized by 13.5% SDS-polyacrylamide gel electrophoresis followed by silver staining. Affinity purified HLA DR3 was obtained by a similar method in 0.01% Tween-80 detergent.

Synthesis of MBP Peptides and Conjugation of Biotin Tag—The *N*-acetylated myelin basic protein peptide analogs MBP(83–102)Y^{83} with the sequence Ac-YDENPVVHFFKNIVTPRTPP and MBP(124–143) peptide with the sequence Ac-GFGYGGRASDYKSAHKGFKG were synthesized by the standard solid phase method using side chain-protected Fmoc (*N*-(9-fluorenyl)methoxycarbonyl) amino acids on an Applied Biosystems 431A automated peptide synthesizer. A tyrosine residue at the N-terminal end of the MBP(83–102)Y^{83} peptide was introduced for radiolabeling of this peptide with ^{125}I. The deprotected, crude peptides were purified by reverse-phase high performance liquid chromatography, and the homogeneity and identity of the purified peptides were confirmed by mass spectrometry. Biotinylation of peptide was carried out as described recently (30).

In Vitro Folding, Reconstitution, and Purification of DR2·MBP Peptide Complexes—Purified α and β polypeptides at a concentration of 0.5 mg/ml were dialyzed against PBS for 18 h at 25 °C. One milligram of α and β chains in the presence of 1–50-fold molar excess of b-MBP(83–102)Y^{83} peptide was incubated for 96 h at 25 °C in an optimized refolding/reconstitution buffer containing 50 mM sodium phosphate (pH 7.5), 1 mM EDTA, 3 mM reduced glutathione, 0.3 mM oxidized glutathione, 25% (v/v) glycerol, and 10 mM dithiothreitol in a total volume of 20 ml. During discovery of the best reconstitution condition, various components from the refolding/reconstitution buffer were deleted one at a time. The complex preparations were dialyzed against 4 liters of PBS at 4 °C with two changes. The reconstituted DR2·MBP peptide complexes were purified by immunoaffinity chromatography using immobilized L243 monoclonal antibody. The column was washed with 10 bed volumes of PBS containing 0.5% Triton X-100 followed by 10 bed volumes of PBS. Finally, bound complexes were eluted in 20 mM phosphate buffer containing 0.1 M NaCl at pH 11.3. Fractions were immediately neutralized by 1 M acetic acid and analyzed by 13.5% silver stain SDS-PAGE under nonreduced conditions.

Characterization of Reconstituted Complexes by Western Blot Analysis and ELISA—Four μg of reconstituted complex were transferred from 13.5% SDS-polyacrylamide gels on polyvinylidene difluoride membranes using a semi-dry transfer cell (Bio-Rad) at 25 V for 20 min. The membranes were incubated for 2 h with anti-DR2 polyclonal serum or purified anti-α and anti-β polyclonal antibodies. The blots were finally developed following a second antibody incubation using 4-chloro-1-naphthol. For the ELISA, 96-well plates (Nunc) were coated with 50 μl of anti-DR2 polyclonal antibody (1:20) in PBS and incubated with 12.5, 25, 50, 100, and 200 ng of reconstituted DR2·b-MBP(83–102)Y^{83} complex for 2 h at 25 °C. The polyclonal antibody-captured complexes were then detected by HRP-coupled L243 monoclonal antibody using TMB as a substrate.

Quantitation of Equimolar Chains in Purified Complexes by Double Label Experiments—Quantitative measurement of the presence of equimolar amounts of both chains in the final complex preparation was assessed by radiolabeling each chain with separate tags. The labeling of the α chain with ^{35}S using a sulfur-labeling reagent (Amersham Corp.) and of the β chain with ^{125}I using Pierce IODO-BEADS was accomplished by previously described methods (31). The specific activity of the ^{35}S-labeled α chain and ^{125}I-labeled β chains were 0.47×10^5 and 3.5×10^5 cpm/μg, respectively.

Detection of Bound Peptide in Reconstituted Complex Preparation—The detection of bound peptide in the purified, reconstituted complex preparations was carried out by incubating equimolar amounts of the two chains in the presence of ^{125}I-labeled b-MBP(83–102)Y^{83} peptide. Radioactive labeling of MBP peptide was achieved by the standard chloramine-T labeling procedure (32). The specific activity of the b-MBP(83–102)Y^{83} was 7.9×10^4 cpm/μg. Equivalent amounts of each α and β chain were incubated with a 10, 50, and 100 molar excess of labeled peptide in 10 ml of refolding/reconstitution buffer at 25 °C for 4 days. Reaction mixtures were extensively dialyzed against PBS and applied to L243 columns. The columns were washed with 10 bed volumes of washing buffer and eluted as described above. The percent of DR2 heterodimer containing labeled peptide was calculated from the specific activity of the peptide.

T Cell Receptor Occupancy Assay—The *Herpesvirus saimiri*-transformed SS8T human T cell clone (33) restricted by DRB5*0101 and MBP(84–102) was cultured in RPMI 1640 medium supplemented with 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 10% fetal bovine serum (HyClone), and 50 units/ml human recombinant interleukin-2 (ABI) at 37 °C. Every alternate day the cells were transferred to fresh medium. Various complex preparations were added at a

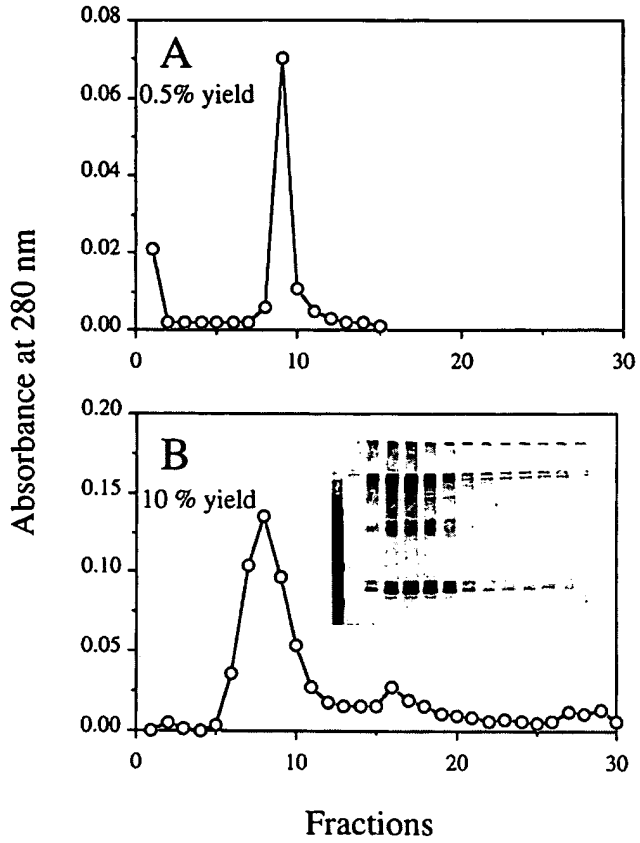

FIG. 1. **Isolation of reconstituted DR2 heterodimer by L243 antibody affinity chromatography.** Elution profiles of reconstituted DR2 heterodimer from unpurified α chain (−TM) and β chain (−TM) inclusion body preparation in the presence of 10-fold molar excess of b-MBP(83–102)Y^{83} peptide is shown in *Panel A*. *Panel B* represents the reconstitution elution profiles of DR2 heterodimers from purified α and β chains in the presence of 10-fold molar excess peptide. The *inset* figure in *Panel B* represents peak fractions analyzed by SDS-PAGE.

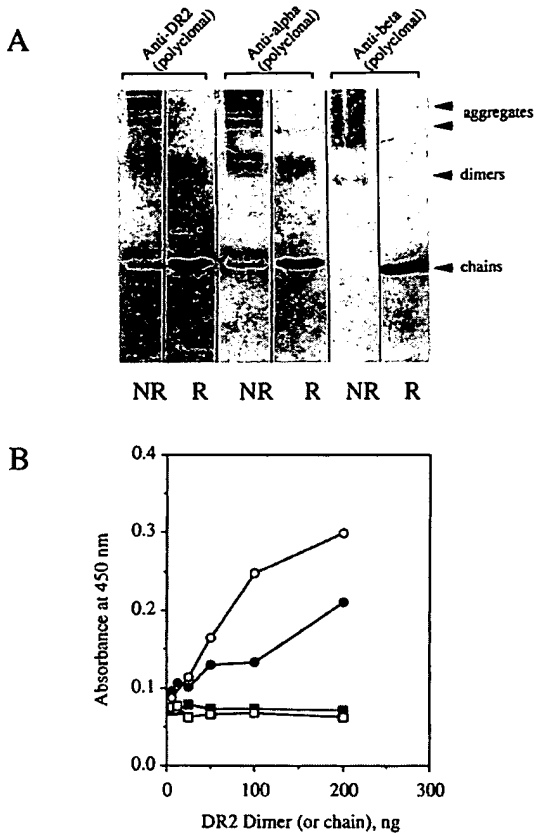

FIG. 2. **Characterization of reconstituted DR2 by Western blot analysis and ELISA.** *Panel A* represents the Western blot analysis of reconstituted DR2 by anti-DR2, anti-α and anti-β polyclonal antibodies, under reduced and nonreduced conditions. *Panel B* represents antibody capture ELISA of heterodimer using dimer specific antibodies. *Closed circles*, native DR2; *open circles*, reconstituted DR2; *closed squares*, α alone; and *open squares*, β alone.

final concentration of 10% v/v in a microtiter tissue culture plate and the cells were inoculated at a density of 20,000/well in 200 μl of medium without interleukin-2. After 48 h of incubation at 37 °C, the supernatants were collected from each well to test for the increase in γ-IFN level. For the detection of γ-IFN, Nunc Maxisorb 96-well plates were coated with anti-human γ-IFN monoclonal antibody at a concentration of 0.5 μg/well and incubated at 4 °C overnight. The wells were blocked with 0.1% bovine serum albumin, and samples were incubated at room temperature for 2 h. The standard curve was generated by using recombinant human γ-IFN with a dilution range of 1000, 500, 100, 50, 10, 5, 1, 0.5, and 0.1 units/ml (270 units/ml = 10.75 ng/ml). Rabbit anti-human γ-IFN was then added at a concentration of 1 μg/ml and plates were incubated at room temperature for an additional 2 h. Wells were extensively washed and incubated with HRP-conjugated goat anti-rabbit at a concentration of 800 ng/ml for 1 h at 37 °C, prior to color developed using TMB as a substrate. The reaction was stopped by 2 N sulfuric acid at 5 min, and the absorbance was measured at 450 nm.

RESULTS AND DISCUSSION

In an attempt to reconstitute human MHC class II heterodimeric protein in the presence of known antigenic peptide and without any endogenously bound peptide, individual α and β chains lacking the transmembrane region were cloned and expressed in an *E. coli* system. The recombinant α and β chains prepared by this method represent approximately 30% of the total cell protein. The insoluble denatured inclusion body preparations were solubilized in 8 M urea and purified in large scale quantities by conventional chromatographic methods as described previously (28).

Individual chains were refolded by slow dialysis at a concentration of 0.5 mg/ml against PBS prior to reconstitution of the heterodimer. Equal amounts of α and β chains were mixed at a concentration of 0.5 mg/ml in the presence of b-MBP(83–102)Y^{83} peptide. The addition of a biotin tag at the N terminus of the peptide was created for the detection of bound MBP peptide in the final complex preparation. In addition, a tyrosine residue at the N terminus of the MBP peptide allowed radiolabeling with ^{125}I. In various quantitative peptide binding studies, the detection of the biotin moiety of the peptide by enzyme conjugated streptavidin resulted in either failure or poor detection of the bound peptide. The reason for an undetected biotin tag in the reconstituted complex preparation is unclear at present and could be due to steric hindrance of the large streptavidin molecule to bind biotin molecules in the peptide binding domain. Based on this unexpected result, the radiolabeled peptide was used to calculate the percent peptide occupancy.

The reconstitution of the heterodimeric molecule in the presence of 10-fold molar excess of b-MBP(83–102)Y^{83} peptide was carried out under several different conditions during optimization of the reconstitution procedure for maximum yield. Among the various conditions, a reconstitution buffer containing both dithiothreitol and glutathione gave the best results. The reconstituted DR2 heterodimers as well as complexes with b-MBP(83–102)Y^{83} peptides were selectively separated from the α and β chains by antibody affinity chromatography using dimer-specific L243 monoclonal antibody. This antibody is known to recognize a monomorphic region of all human HLA

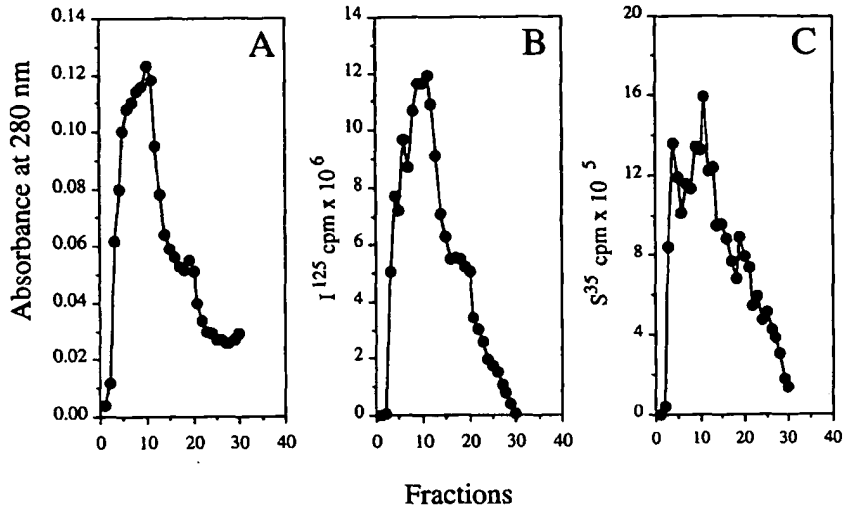

FIG. 3. **Elution of double-labeled reconstituted DR2 heterodimer from L243 column.** α and β chains were radiolabeled with ^{35}S and ^{125}I, respectively, as described under "Materials and Methods." Purified labeled chains were allowed to refold in the presence of 10-fold molar excess of unlabeled b-MBP(83–102)Y^{83} peptide. *Panel A* represents the elution profile of reconstituted DR2 by absorbency at 280 nm. *Panel B* represents the profile of radiolabeled ^{125}I of β chain, as measured by the γ radiation count. *Panel C* represents the profile of radiolabeled ^{35}S of α chain as measured by the β radiation count.

DR molecules and the epitope for this monoclonal antibody has been mapped on the α chain of the DR molecule (34). The lack of reactivity of the L243 antibody to individual α and β chains by ELISA and Western blot analyses was also confirmed in our laboratory (data not shown). In various control experiments, we observed that the α chain alone incubated in the presence of 10-fold molar excess of the b-MBP(83–102)Y^{83} peptide showed very weak binding to the L243 antibody column and the eluted minor peak represents less than 2% of the total eluted heterodimer preparation. In contrast, incubation of the β chain in the presence of 10-fold molar excess of the b-MBP(83–102)Y^{83} peptide showed absolutely no binding to the L243 monoclonal antibody. Since individual chains are known to form a low level of α-α and β-β homodimers in solution (35, 36), the small fraction of α chain binding to L243 monoclonal antibody affinity column could be due to the formation of a minor fraction of α-α homodimers in solution.

The reconstitution of functionally active heterodimers carried out from crude unpurified preparations of α and β chains showed substantially low yield as compared to purified chains. As shown in Fig. 1, reconstitution performed with purified chains in the presence of equal amount of peptide resulted in 20-fold increase in yield as compared to unpurified chains. Eluted peak fractions were analyzed by SDS-PAGE which showed the presence of both heterodimers, partially dissociated monomers along with various levels of aggregates (see Fig. 1*B*, *inset*). Like the native heterodimer, various levels of aggregation were also observed for reconstituted heterodimer under nonreduced gel electrophoresis conditions. Upon reduction, these bands of high molecular mass merged into the expected molecular sizes of 21 and 23 kDa for two chains. The unexpected high molecular aggregates of purified complexes lacking the transmembrane hydrophobic regions suggest that the aggregation level of various purified native MHC class II molecules observed consistently is not dependent upon the hydrophobic transmembrane regions of these molecules. Using dynamic multi-angle laser light scattering measurements, we observed that the peptide plays a critical role in destabilizing such aggregates of MHC class II molecules,[2] suggesting that most of the heterodimer aggregates observed in our reconstituted samples are the populations containing no bound peptide.

The presence of both α and β polypeptide chains in the eluted, reconstituted peak was demonstrated by Western blot analysis and ELISA. Western blot analysis was performed using heterodimer and chain-specific polyclonal antibodies. As shown in Fig. 2*A*, the reconstituted heterodimer was recognized by all three polyclonal antibodies under both reduced and nonreduced conditions. Similarly, the presence of both α and β chains was characterized by antibody capture ELISA (Fig. 2*B*). In this assay, the reconstituted and native DR2 were captured by heterodimer-specific polyclonal antibody and detected by HRP-conjugated, L243-purified monoclonal antibody. The α chain and the β chain alone, incubated with equivalent amounts of b-MBP(83–102)Y^{83} peptide, did not show any reactivity in this assay.

To demonstrate the presence of two chains in equimolar amounts, the α and β chains were separately radiolabeled with ^{35}S and ^{125}I, respectively. The selection of the labeling tag for each chain was based on their sequences. The α chain contains 9 lysine residues whereas the β chain contains 9 tyrosine residues. Equimolar amounts of the two differently labeled chains were refolded and mixed with 10-fold molar excess amount of b-MBP(83–102)Y^{83} peptide. The reconstituted heterodimers were eluted from the L243 monoclonal antibody affinity column and analyzed for the presence of two chains. Fig. 3 shows the absorbance of eluted fractions along with ^{125}I and ^{35}S counts of each fraction measured in γ and β counters, respectively. The quantitation of each chain in the final eluted pool was carried out using the specific activities of the two chains. Results presented in Table I show that both chains are present in almost equal amounts. A small increase in the total α chain correlates well with our observation that a small fraction of α-α homodimers are capable of binding L243 antibody.

The effect of antigenic peptide concentration on the yield of reconstituted heterodimers was next examined by incubating equimolar amounts of the two chains in the presence of increasing peptide concentrations. As shown in Fig. 4, increasing the peptide concentration has a significant effect on the yield of reconstituted DR2 heterodimers as seen by the elution profile and SDS-PAGE analysis of peak fractions. At a peptide concentration of 50-fold molar excess, the yield of reconstituted DR2 was more than 20% (Fig. 4). We observed a further increase in yield up to 29% with 100-fold molar excess of the MBP peptide. Thus, the presence of peptide is critical in increasing the yield of reconstituted complexes. A low level of reconstituted DR2 heterodimer representing 1–3% of the starting protein was also observed in various experiments in the absence of b-MBP(83–102)Y^{83} peptide. The peptide-dependent refolding and assembly of complexes correlates well with various earlier reports with MHC class I refolding (37, 38) *in vitro*. *In vivo*, the

[2] B. Nag, unpublished results.

TABLE I
Double-labeled refolding experiment

Purified α chain was ^{35}S-radiolabeled by sulfur-labeling reagent (SLR) at various lysine residues, and the β chain was labeled by IODO-BEADS using ^{125}I. Equimolar amounts of both α and β chains were incubated in the presence of 10-fold molar excess b-MBP(83–102) Y^{83} peptide. Heterodimeric complexes were separated on L243 immobilized Sepharose 4B column.

Chains	Molecular weight	Radiolabel tag	Labeled residue	Specific activity	Starting protein	Post-antibody	Post-antibody total protein	Chains
				cpm/μg	*μg*	*Total cpm*	*μg*	*mM*
α (−TM)	21,109	^{35}S (SLR)	9 lysine	0.47×10^5	748	1.5×10^7	319	15.1
β (−TM)	23,024	^{125}I (IODO-BEADS)	9 tyrosine	3.5×10^5	760	1.17×10^8	334	14.5

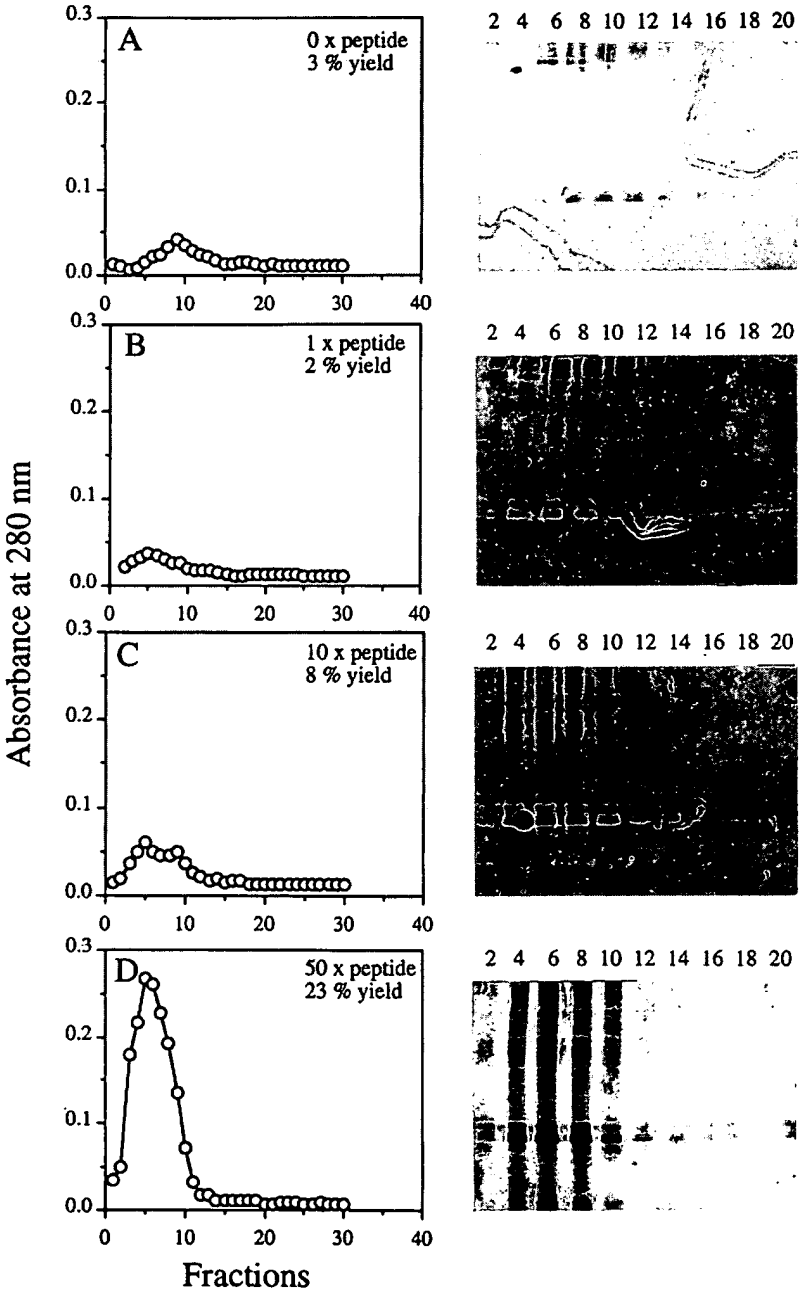

FIG. 4. **Effect of the peptide concentration on reconstitution of heterodimers.** Equivalent amounts of α and β chains were incubated with no peptide (*Panel A*), 1-fold molar excess (*Panel B*), 10-fold molar excess (*Panel C*), and 50-fold molar excess (*Panel D*) b-MBP(83–102)Y^{83} peptide. Reconstituted DR2 complexes were purified over L243 antibody column. Fractions of each elution profile (10 μl) were analyzed by 13.5% SDS-PAGE followed by silver staining.

presence of peptide is also a prerequisite for the correct folding and transport of MHC class II-peptide complexes to the surface of the antigen-presenting cells (39–44). In this regard, peptides are also known to stabilize the quaternary structure of MHC class II molecules (45).

The quantitation of bound peptide in the refolded and reconstituted DR2 heterodimer preparation was carried out by increasing concentrations of radiolabeled peptide. The b-MBP(83–102)Y^{83} peptide was radiolabeled by ^{125}I using the chloramine-T method and was incubated at various concentrations with equivalent amount of purified α and β chains. Although increasing the peptide concentration had significant

TABLE II

Percent of reconstituted complex containing MBP peptide

Equivalent amount of two chains were mixed with ^{125}I-labeled b-MBP (83–102)Y^{83} peptide and reconstituted complexes were purified by L243 antibody column. The specific activity of the b-MBP(83–102)Y^{83} peptide was 7.9×10^4 cpm/μg.

α chain	β chain	Peptide molar excess	Binding
mg			%
0.5	0.5	10	17.0
0.5	0.5	50	18.0
0.5	0.5	100	18.6

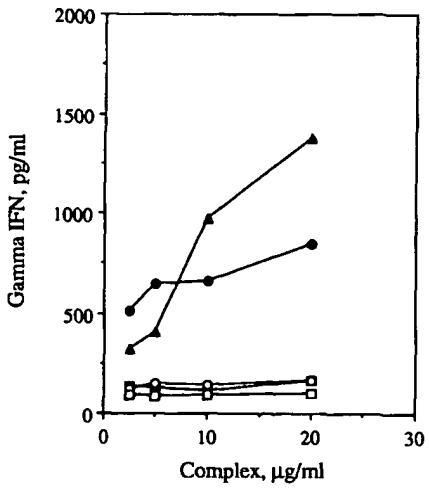

FIG. 5. **Level of γ-IFN production of SS8T T cells in the presence of reconstituted DR2·b-MBP(83–102)Y^{83} complex.** Occupancy of TCR by complexes of reconstituted DR2. b-MBP(83–102)Y^{83} was shown by increased level of γ IFN using SS8T transformed T cells, restricted to DR2 and MBP(84–102) complexes. Purified native DR2 and DR3 proteins at a concentration of 0.5 mg/ml were incubated with 50-fold molar excess of respective peptides in citrate buffer pH 6.0 at 37 °C for 96 h. The complexes of DR2·b-MBP(83–102)Y^{83}, DR2·b-MBP(124–143), and DR3·b-MBP(83–102)Y^{83} were prepared and the unbound free peptide was removed by passing through Sephadex G-75 gel filtration column. The level of γ-IFN was measured by ELISA as described under "Material and Methods." *Closed circles*, native DR2·b-MBP (83–102)Y^{83} complex; *open circles*, DR2 alone; *open squares*, DR2 with irrelevant b-MBP(124–143) peptide; *closed squares*, DR3 with b-MBP(83–102)Y^{83}; and *closed triangles*, reconstituted DR2·b-MBP(83–102)Y^{83} complex.

effect on the yield of reconstituted DR2 heterodimer, the percent of DR2 occupied with bound peptide remained constant (Table II).

Finally, the functional activity of reconstituted heterodimeric complexes was analyzed by measuring the increased level of γ-IFN produced by *H. saimiri*-transformed SS8T cloned T cells in a dose-dependent manner. An increase in γ-IFN production has been correlated with the occupancy of TCR on the surface of T cells (33). The specificity and restriction of the SS8T transformed T cell clone for the HLA DR2 and MBP(84–102) peptide has been demonstrated recently (33). As shown in Fig. 5, both native and reconstituted DR2·b-MBP(83–102)Y^{83} complexes were able to recognize TCR on the surface of transformed T cells. The reconstituted DR2·b-MBP(83–102)Y^{83} complexes appeared to be more effective in producing γ-IFN than native DR2·b-MBP(83–102)Y^{83} complexes in several experiments (data not shown). In various controls, DR2 alone, DR2 with irrelevant peptide b-MBP(124–143) and b-MBP(83–102)Y^{83} peptide complexed with irrelevant MHC class II (HLA DR3), showed no significant increase in the level of γ-IFN production. Human T cells are known to express low levels of MHC class II molecules on their surfaces and can be stimulated in the presence of antigenic peptide (46, 47). In order to demonstrate that the observed level of increased γ-IFN is not due to the release of bound peptide in the culture medium, the b-MBP(83–102)Y^{83} peptide was complexed with HLA DR3 as a control which showed no increase in γ-IFN level. Similarly in mock experiment equivalent amount of b-MBP(83–102)Y^{83} peptide incubated under identical refolding conditions in the absence of chains did not show any increase in γ-IFN level.

In conclusion, results presented here demonstrate the formation of functionally active HLA DR2 heterodimeric complexes containing antigenic epitopes. The yield of such complexes is approximately 8000-fold higher than the native DR2 molecules. Present results from our laboratory showed that purified MHC class II-peptide complexes can be used for the prevention and treatment of experimental autoimmune disease in various animal models[3,4] (48). The reconstituted heterodimeric MHC class II containing known antigenic epitope may have significant clinical relevance in developing antigen-specific therapeutics for various autoimmune diseases. In addition, such complexes in sufficient quantities, will facilitate several structure-function studies including x-ray crystallographic studies for better understanding of the trimolecular interactions among MHC class II, peptide, and TCR.

Acknowledgments—We thank Dr. Shrikant Deshpande for providing various MBP peptides, Dr. H. Wekerle for providing SS8T transformed T cell clone, Dr. Chris Raymond for providing the polyclonal antibodies against native DR2 heterodimer, and Dr. Jeffery Winkelhake for critically reading the manuscript.

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L2            16 S L1 AND MHC
L3            12 DUP REM L2 (4 DUPLICATES REMOVED)
L4            62 S (SINGLE (1N) CHAIN) (10N) (CLASS (1N) II)
L5            30 DUP REM L4 (32 DUPLICATES REMOVED)
L6             2 S L5 AND PD<19960131
L7            28 S L5 NOT L6
L8           766 S TETRAMER (P) MHC
L9            23 S L8 AND PD<19960131
L10           11 DUP REM L9 (12 DUPLICATES REMOVED)

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 NEWS  6  Mar 08  Gene Names now available in BIOSIS
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 NEWS  8  Mar 22  TRCTHERMO no longer available
 NEWS  9  Mar 28  US Provisional Priorities searched with P in CA/CAplus
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=> s Rhode P?/au or Jiao J?/au or Burkhardt M?/au or Wong H?/au
L1        4867 RHODE P?/AU OR JIAO J?/AU OR BURKHARDT M?/AU OR WONG H?/AU

=> s l1 and MHC
L2          16 L1 AND MHC

=> dup rem l2
PROCESSING COMPLETED FOR L2
L3          12 DUP REM L2 (4 DUPLICATES REMOVED)

=> dis l3 1-12 ibib abs kwic

L3   ANSWER 1 OF 12  CAPLUS  COPYRIGHT 2002 ACS
ACCESSION NUMBER:        2001:868803  CAPLUS
DOCUMENT NUMBER:         135:370658
TITLE:                   Modulation of T-cell receptor interactions
INVENTOR(S):             Rhode, Peter; Wittman, Vaughan; Weidanz, Jon
                         A.; Burkhardt, Martin; Card, Kimberlyn F.;
                         Tal, Rony; Acevedo, Jorge; Wong, Hing C.
PATENT ASSIGNEE(S):      Sunol Molecular Corporation, USA
SOURCE:                  PCT Int. Appl., 207 pp.
                         CODEN: PIXXD2
DOCUMENT TYPE:           Patent
LANGUAGE:                English
FAMILY ACC. NUM. COUNT:  1
PATENT INFORMATION:

     PATENT NO.        KIND  DATE           APPLICATION NO.  DATE
     ----------------  ----  --------       ---------------  --------
     WO 2001090747     A2    20011129       WO 2001-US15699  20010516
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
             CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM,
             HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS,
             LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO,
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RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
PRIORITY APPLN. INFO.: US 2000-206920P P 20000525
AB Disclosed are methods for identifying compds. that modulate the interaction between T cell receptors (TCR) and major histocompatibility complex (MHC) antigens. The invention has many useful applications including providing high throughput screening assays for detecting compns. that can modulate an immune response.
IN **Rhode, Peter**; Wittman, Vaughan; Weidanz, Jon A.; **Burkhardt, Martin**; Card, Kimberlyn F.; Tal, Rony; Acevedo, Jorge; **Wong, Hing C.**
AB Disclosed are methods for identifying compds. that modulate the interaction between T cell receptors (TCR) and major histocompatibility complex (MHC) antigens. The invention has many useful applications including providing high throughput screening assays for detecting compns. that can modulate an immune response.
ST TCR MHC complex interaction immunomodulator antibody
IT Immunoglobulins
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(A, hybrid with MHC mols.; methods for identifying compds. that modulate the interaction between T cell receptors and major histocompatibility complex antigens)
IT Immunoglobulins
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(G, hybrid with MHC mols.; methods for identifying compds. that modulate the interaction between T cell receptors and major histocompatibility complex antigens)
IT Immunoglobulins
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(M, hybrid with MHC mols.; methods for identifying compds. that modulate the interaction between T cell receptors and major histocompatibility complex antigens)
IT Histocompatibility antigens
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(MHC (major histocompatibility complex), class I, peptide complexes; methods for identifying compds. that modulate the interaction between T cell receptors and major histocompatibility complex antigens in relation to)
IT Histocompatibility antigens
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(MHC (major histocompatibility complex), class II, peptide complexes; methods for identifying compds. that modulate the interaction between T cell receptors and major histocompatibility complex antigens in relation to)
IT Histocompatibility antigens
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(MHC (major histocompatibility complex), peptide comlex; methods for identifying compds. that modulate the interaction between T cell receptors and major histocompatibility complex antigens)
IT Histocompatibility antigens
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(MHC (major histocompatibility complex); methods for identifying compds. that modulate the interaction between T cell receptors and major histocompatibility complex antigens)
IT Glycolipids
Lipids, biological studies
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(MHC complexes; methods for identifying compds. that modulate the interaction between T cell receptors and major histocompatibility complex antigens)
IT Antigens
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(superantigens, MHC complexes; methods for identifying compds. that modulate the interaction between T cell receptors and major histocompatibility complex antigens)
IT CD3 (antigen)
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(.zeta. chain; chimera with MHC mols.; methods for identifying compds. that modulate the interaction between T cell receptors and major histocompatibility complex antigens)

L3 ANSWER 2 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:6971 BIOSIS
DOCUMENT NUMBER: PREV200200006971
TITLE: MHC molecules and uses thereof.
AUTHOR(S): **Rhode, Peter R.; Jiao, Jin-An (1); Burkhardt, Martin; Wong, Hing C.**
CORPORATE SOURCE: (1) Fort Lauderdale, FL USA
ASSIGNEE: Sunol Molecular Corporation
PATENT INFORMATION: US 6309645 October 30, 2001
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Oct. 30, 2001) Vol. 1251, No. 5, pp. No Pagination. e-file.
ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
AB The present invention relates to novel complexes of major histocomability complex (MHC) molecules and uses of such complexes. In one aspect, the invention relates to loaded MHC complexes that include at least one MHC molecule with a peptide-binding groove and a presenting peptide non-covalently linked to the MHC

protein. In another aspect, the invention features single chain MHC class II peptide fusion complexes with a presenting peptide covalently linked to the peptide binding grove of the complex. MHC complexes of the invention are useful for a variety of applications including: 1) in vitro screens for identification and isolation of peptides that modulate activity of selected T cells, including peptides that are T cell receptor antagonists and partial agonists, and 2) methods for suppressing or inducing an immune response in a mammal.

TI MHC molecules and uses thereof.

AU **Rhode, Peter R.; Jiao, Jin-An (1); Burkhardt, Martin; Wong, Hing C.**

AB The present invention relates to novel complexes of major histocomability complex (MHC) molecules and uses of such complexes. In one aspect, the invention relates to loaded MHC complexes that include at least one MHC molecule with a peptide-binding groove and a presenting peptide non-covalently linked to the MHC protein. In another aspect, the invention features single chain MHC class II peptide fusion complexes with a presenting peptide covalently linked to the peptide binding grove of the complex. MHC complexes of the invention are useful for a variety of applications including: 1) in vitro screens for identification and isolation. . .

IT Major Concepts
Clinical Immunology (Human Medicine, Medical Sciences); Pharmacology

IT Chemicals & Biochemicals
major histocompatibility complex molecules (MHC molecules): immunologic - drug

L3 ANSWER 3 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:499745 BIOSIS
DOCUMENT NUMBER: PREV200100499745
TITLE: Soluble MHC complexes and methods of use thereof.
AUTHOR(S): **Rhode, Peter R.**; Acevedo, Jorge (1); **Burkhardt, Martin; Jiao, Jin-an; Wong, Hing C.**
CORPORATE SOURCE: (1) Miami, FL USA
ASSIGNEE: Sunol Molecular Corporation
PATENT INFORMATION: US 6232445 May 15, 2001
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (May 15, 2001) Vol. 1246, No. 3, pp. No Pagination. e-file.
ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English

AB The present invention relates to novel complexes of major histocompability complex (MHC) molecules and uses of such complexes. In one aspect, the invention relates to single chain MHC class II complexes that include a class II beta2 chain modification, e.g., deletion of essentially the entire class II beta2 chain. In another aspect, the invention features single chain MHC class II which comprise an immunoglobin constant chain or fragment. Further provided are polyspecific MHC complexes comprising at least one single chain MHC class II molecule. MHC complexes of the invention are useful for a variety of applications including: 1) in vitro screens for identification and isolation of peptides that modulate activity of selected T cells, including peptides that are T cell receptor antagonists and partial agonists, and 2) methods for suppressing or inducing an immune response in a mammal.

TI Soluble MHC complexes and methods of use thereof.

AU **Rhode, Peter R.**; Acevedo, Jorge (1); **Burkhardt, Martin ; Jiao, Jin-an; Wong, Hing C.**

AB The present invention relates to novel complexes of major histocompability complex (MHC) molecules and uses of such complexes. In one aspect, the invention relates to single chain MHC class II complexes that include a class II beta2 chain modification, e.g., deletion of essentially the entire class II beta2 chain. In another aspect, the invention features single chain MHC class II which comprise an immunoglobin constant chain or fragment. Further provided are polyspecific MHC complexes comprising at least one single chain MHC class II molecule. MHC complexes of the invention are useful for a variety of applications including: 1) in vitro screens for identification and isolation. . .

IT Major Concepts
Pharmacology

IT Chemicals & Biochemicals
soluble MHC complexes: immunologic - drug, method of use

L3 ANSWER 4 OF 12 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:277860 CAPLUS
DOCUMENT NUMBER: 132:320940
TITLE: Polyspecific binding molecules and uses thereof
INVENTOR(S): Weidanz, Jon A.; Card, Kimberlyn; Sherman, Linda A.; Klinman, Norman R.; **Wong, Hing C.**
PATENT ASSIGNEE(S): Sunol Molecular Corporation, USA
SOURCE: PCT Int. Appl., 130 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000023087	A1	20000427	WO 1999-US24645	19991021
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1124568	A1	20010822	EP 1999-970601	19991021
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				

PRIORITY APPLN. INFO.: US 1998-105164P P 19981021
WO 1999-US24645 W 19991021

AB The present invention relates to polyspecific binding mols. and particularly single-chain polyspecific binding mols. that include at least one single-chain T-cell receptor (s.c.-TCR) covalently linked through a peptide linker sequence to at least one single-chain antibody (s.c.-Ab). The polyspecific binding mols. activate immune cells (e.g. cytotoxic T cells, NK cells or macrophages) and kill target cells (e.g. tumor cells or

virally infected cells). The polyspecific binding mols. are useful for diagnosis and treatment of cancers and viral infections.
REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT
IN Weidanz, Jon A.; Card, Kimberlyn; Sherman, Linda A.; Klinman, Norman R.; **Wong, Hing C.**
IT Histocompatibility antigens
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(MHC (major histocompatibility complex); polyspecific binding mols. comprising single chain TCR and Ig for diagnosis and therapy of tumor or viral infection)

L3 ANSWER 5 OF 12 MEDLINE
ACCESSION NUMBER: 2000442218 MEDLINE
DOCUMENT NUMBER: 20443576 PubMed ID: 10990169
TITLE: Immune cell signaling in lupus.
AUTHOR: Tsokos G C; **Wong H K**; Enyedy E J; Nambiar M P
CORPORATE SOURCE: Department of Medicine, Uniformed Services University of the Health Sciences, and Walter Reed Army Institute of Research, Silver Spring, Maryland 20910-7500, USA.. gtsokos@usa.net
CONTRACT NUMBER: AI 422269 (NIAID)
SOURCE: CURRENT OPINION IN RHEUMATOLOGY, (2000 Sep) 12 (5) 355-63. Ref: 60
Journal code: AVG. ISSN: 1040-8711.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200101
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010104
AB The fate of the lymphocyte is determined by integration of signals delivered after the binding of antigen to the surface antigen receptor, signals delivered by cytokines that bind to their surface receptors, and signals initiated after the engagement of other surface receptors, known as costimulatory molecules. The summation of this input determines whether the immune cell will become stimulated, ignore the signal (anergy), or die (apoptosis). Antigen-receptor signaling events are abnormal in lupus lymphocytes, manifested by increased calcium responses and hyperphosphorylation of several cytosolic protein substrates. Further down, at the gene transcription level, the activity of the nuclear factor kappaB is decreased. These events are underwritten by defective T cell receptor zeta chain expression, overexpression of the gamma chain of the Fc(epsilon)RI that functions as an alternate of zeta chain, and decreased p65 -Rel A protein that is responsible for the inducible NFkappaB activity. Accumulated research data have enabled us to begin deciphering the molecular basis of the abnormal lupus lymphocyte and may lead to the development of new medicinal treatments for lupus.
AU Tsokos G C; **Wong H K**; Enyedy E J; Nambiar M P
CT . . . Animal; Human; Support, U.S. Gov't, P.H.S.
B-Lymphocytes: PH, physiology
Cell Cycle
Cyclin-Dependent Kinases: AI, antagonists & inhibitors
Estrogens: PH, physiology
Genes, MHC Class II: PH, physiology
*Lupus Erythematosus, Systemic: GE, genetics
*Lupus Erythematosus, Systemic: IM, immunology
Lupus Erythematosus, Systemic: PP,. . .

L3 ANSWER 6 OF 12 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:297317 CAPLUS
DOCUMENT NUMBER: 130:295539
TITLE: Construction of chimeric soluble **MHC** complexes
INVENTOR(S): **Rhode, Peter R.**; Acevedo, Jorge; **Burkhardt, Martin**; **Jiao, Jin-an**; **Wong, Hing C.**
PATENT ASSIGNEE(S): Sunol Molecular Corporation, USA
SOURCE: PCT Int. Appl., 148 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9921572	A1	19990506	WO 1998-US21520	19981013
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6232445	B1	20010515	US 1997-960190	19971029
CA 2307178	AA	19990506	CA 1998-2307178	19981013
AU 9898001	A1	19990517	AU 1998-98001	19981013
EP 1027066	A1	20000816	EP 1998-952256	19981013
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2002508300	T2	20020319	JP 2000-517730	19981013
PRIORITY APPLN. INFO.:			US 1997-960190 A	19971029
			WO 1998-US21520 W	19981013

AB The authors disclose the construction and expression of sol. single-chain (s.c.) **MHC** class II mols. In one aspect, the s.c.-**MHC** class II mols. include a .beta.2 chain modification, e.g., deletion of essentially the entire class II .beta.2 domain. In another aspect, the invention features single-chain **MHC** class II which contain an Ig light chain const. region fragment (CL). The CL fragment allows multimerization of single-chain monomers of identical or disparate **MHC** specificity or formation of heteromeric mols. with effector function (e.g., single-chain antibodies). In addn., the sol. **MHC** class II mols. can be constructed for exogenous loading of cognate peptides or the requisite peptides can be included in the single-chain constructs themselves. In one example, single-chain I-Ad mols. were constructed as fusion proteins with T-cell epitopes from either ovalbumin or glycoprotein D of herpes simplex virus. These constructs were shown to

stimulate interleukin-2 prodn. by their resp. antigen-specific T-cells. MHC complexes of the invention are useful for a variety of applications including: (1) in vitro screens for identification and isolation of peptides that modulate activity of selected T-cells, including peptides that are T cell receptor antagonists and partial agonists, and (2) methods for suppressing or inducing an immune response in a mammal.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI Construction of chimeric soluble MHC complexes

IN **Rhode, Peter R.**; Acevedo, Jorge; **Burkhardt, Martin**; **Jiao, Jin-an**; **Wong, Hing C.**

AB The authors disclose the construction and expression of sol. single-chain (s.c.) MHC class II mols. In one aspect, the s.c.-MHC class II mols. include a .beta.2 chain modification, e.g., deletion of essentially the entire class II .beta.2 domain. In another aspect, the invention features single-chain MHC class II which contain an Ig light chain const. region fragment (CL). The CL fragment allows multimerization of single-chain monomers of identical or disparate MHC specificity or formation of heteromeric mols. with effector function (e.g., single-chain antibodies). In addn., the sol. MHC class II mols. can be constructed for exogenous loading of cognate peptides or the requisite peptides can be included in the single-chain constructs themselves. In one example, single-chain I-Ad mols. were constructed as fusion proteins with T-cell epitopes from either ovalbumin or glycoprotein D of herpes simplex virus. These constructs were shown to stimulate interleukin-2 prodn. by their resp. antigen-specific T-cells. MHC complexes of the invention are useful for a variety of applications including: (1) in vitro screens for identification and isolation of peptides that modulate activity of selected T-cells, including peptides that are T cell receptor antagonists and partial agonists, and (2) methods for suppressing or inducing an immune response in a mammal.

ST soluble histocompatibility class II antigen fusion protein; single chain MHC class II soluble fusion protein

IT Immunoglobulin fragments
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); PRP (Properties); BIOL (Biological study); PREP (Preparation)
(CL, fusion products with single-chain MHC class II mols.; prepn., enhanced soly., and biol. activity of)

IT Synthetic genes
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(animal; for expression of sol. single-chain MHC class II mols.)

IT Immunosuppression
(by sol. single-chain MHC class II mols.)

IT Genetic vectors
(for expression of sol. single-chain MHC class II mols.)

IT Peptides, biological studies
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); PRP (Properties); BIOL (Biological study); PREP (Preparation)
(fusion peptides, with single-chain MHC class II mols.; prepn. and biol. activity of)

IT Class II MHC antigens
I-Ad antigen
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); PRP (Properties); BIOL (Biological study); PREP (Preparation)
(fusion products, with antigenic peptides; prepn. and biol. activity of sol. single-chain constructs of)

IT Immunoglobulin light chains
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); PRP (Properties); BIOL (Biological study); PREP (Preparation)
(fusion products, with single-chain MHC class II mols.; prepn., enhanced soly., and biol. activity of)

IT Transformation (genetic)
(of host cells for expression of sol. single-chain MHC class II mols.)

IT Solubility
(of single-chain MHC class II mols. fused to Ig C.kappa. light chain fragments)

IT Fusion proteins (chimeric proteins)
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); PRP (Properties); BIOL (Biological study); PREP (Preparation)
(of sol. single-chain MHC class II heterodimers with, or without, fusion to T-cell epitopes and/or Ig light chain const. region fragments)

IT Genetic engineering
(of sol. single-chain MHC class II mols.)

IT Mouse
(prepn., enhanced soly., and biol. activity of single-chain MHC class II mols. fused to Ig light chain fragment of)

IT T cell (lymphocyte)
(sol. single-chain MHC class II mols. modulate response by)

IT Genes (animal)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(synthetic; for expression of sol. single-chain MHC class II mols.)

IT Peptide complexes
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(with single-chain MHC class II mols.; prepn. and biol. activity of)

IT 56-45-1, L-Serine, biological studies
RL: BOC (Biological occurrence); BIOL (Biological study); OCCU (Occurrence)
(residue 117; substitution for cysteine in .beta.2 domain of sol. single-chain MHC class II mols.)

L3 ANSWER 7 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:246173 BIOSIS
DOCUMENT NUMBER: PREV199900246173
TITLE: Single chain MHC complexes and uses thereof.
AUTHOR(S): **Rhode, P. R.**; **Jiao, J-A.**; **Burkhardt, M.**; **Wong, H. C.**
CORPORATE SOURCE: Miami, Fla. USA
ASSIGNEE: SUNOL MOLECULAR CORPORATION
PATENT INFORMATION: US 5869270 Feb. 9, 1999
SOURCE: Official Gazette of the United States Patent and Trademark

Office Patents, (Feb. 9, 1999) Vol. 1219, No. 2, pp. 1524.
ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
TI Single chain MHC complexes and uses thereof.
AU **Rhode, P. R.; Jiao, J-A.; Burkhardt, M.; Wong, H. C.**
IT Miscellaneous Descriptors
BIOTECHNOLOGY; MAJOR HISTOCOMPATIBILITY COMPLEX; MHC; SINGLE CHAIN CLASS II MHC COMPLEX

L3 ANSWER 8 OF 12 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:618856 CAPLUS
DOCUMENT NUMBER: 129:229693
TITLE: Fusion proteins comprising bacteriophage coat protein and a single-chain T cell receptor
INVENTOR(S): Weidanz, Jon A.; Card, Kimberlyn F.; **Wong, Hing C.**
PATENT ASSIGNEE(S): Sunol Molecular Corporation, USA
SOURCE: PCT Int. Appl., 151 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9839482	A1	19980911	WO 1998-US4274	19980305
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9866856	A1	19980922	AU 1998-66856	19980305
EP 977886	A1	20000209	EP 1998-908950	19980305
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2001514503	T2	20010911	JP 1998-537984	19980305

PRIORITY APPLN. INFO.: US 1997-813781 A 19970307
WO 1998-US4274 W 19980305

AB The present invention relates to novel fusion proteins comprising a bacteriophage coat protein and a single-chain T cell receptor and uses of such complexes. In one aspect, the invention relates to sol. fusion protein comprising a bacteriophage coat protein covalently linked to a single-chain T cell receptor which comprises a V-.alpha. gene covalently linked to a V-.beta. chain by a peptide linker sequence. The single-chain TCR fusion protein typically also includes one or more fused protein tags to help purify the fusion protein from cell components which can accompany it. The TCR used was murine DO11.10 cell TCR which recognizes and binds a chicken ovalbumin peptide spanning amino acids 323-339 in the context of an I-Ad MHC class II mol. The sol. fusion proteins of the invention are useful for a variety of applications including: (1) making a bacteriophage library for displaying single-chain T cell receptors for use in screens for identification and isolation of ligands that bind single-chain T cell receptors, and (2) methods for isolating sol. and fully functional single-chain T cell receptors from the fusion proteins. The single-chain TCR fusion proteins can be made without performing difficult solubilization, protein refolding or cleaving steps; formation of inclusion bodies in expressing cells is minimal, thereby significantly increasing yields.
IN Weidanz, Jon A.; Card, Kimberlyn F.; **Wong, Hing C.**
AB The present invention relates to novel fusion proteins comprising a bacteriophage coat protein and a single-chain T cell receptor and uses of such complexes. In one aspect, the invention relates to sol. fusion protein comprising a bacteriophage coat protein covalently linked to a single-chain T cell receptor which comprises a V-.alpha. gene covalently linked to a V-.beta. chain by a peptide linker sequence. The single-chain TCR fusion protein typically also includes one or more fused protein tags to help purify the fusion protein from cell components which can accompany it. The TCR used was murine DO11.10 cell TCR which recognizes and binds a chicken ovalbumin peptide spanning amino acids 323-339 in the context of an I-Ad MHC class II mol. The sol. fusion proteins of the invention are useful for a variety of applications including: (1) making a bacteriophage library for displaying single-chain T cell receptors for use in screens for identification and isolation of ligands that bind single-chain T cell receptors, and (2) methods for isolating sol. and fully functional single-chain T cell receptors from the fusion proteins. The single-chain TCR fusion proteins can be made without performing difficult solubilization, protein refolding or cleaving steps; formation of inclusion bodies in expressing cells is minimal, thereby significantly increasing yields.

L3 ANSWER 9 OF 12 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 1999110189 MEDLINE
DOCUMENT NUMBER: 99110189 PubMed ID: 9894898
TITLE: Display of functional alphabeta single-chain T-cell receptor molecules on the surface of bacteriophage.
AUTHOR: Weidanz J A; Card K F; Edwards A; Perlstein E; **Wong H C**
CORPORATE SOURCE: Sunol Molecular, Miramar, FL 33025, USA.. jaweid@laker.net
CONTRACT NUMBER: R43-CA76856-01 (NCI)
SOURCE: JOURNAL OF IMMUNOLOGICAL METHODS, (1998 Dec 1) 221 (1-2) 59-76.
Journal code: IPE; 1305440. ISSN: 0022-1759.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199901
ENTRY DATE: Entered STN: 19990216
Last Updated on STN: 19990216
Entered Medline: 19990129

AB The ability to display functional T-cell receptors (TCR) on the surface of bacteriophage could have numerous applications. For instance, TCR phage-display could be used to develop new strategies for isolating TCRs with unique specificity or it could be used to carry out mutagenesis studies on TCR molecules for analyzing their structure-function. We initially selected a TCR from the murine T-cell hybridoma, DO11.10, as our model system, and genetically engineered a three domain single-chain TCR

(scTCR) linked to the gene p8 protein of the Escherichia coli bacteriophage fd. Immunoblotting studies revealed that (1) E. coli produced a soluble scTCR/p8 fusion protein and (2) the fusion protein was packaged by the phage. Cellular competition assays were performed to evaluate the functionality of the TCR and showed the DO11.10 TCR-bearing phage could significantly inhibit stimulation of DO11.10 T hybridoma cells by competing for binding to immobilized MHC/peptide IA(d)/OVA(323-339). Flow cytometric analysis was carried out to evaluate direct binding of DO11.10 TCR-bearing phage onto the surface of cells displaying either IAd containing irrelevant peptide or OVA peptide. The results revealed binding of DO11.10 TCR-bearing phage only on cells expressing IA(d) loaded with OVA peptide showing TCR fine specificity for peptide. To illustrate the generality of TCR phage-display, we also cloned and displayed on phage a second TCR which recognizes a peptide fragment from human tumor suppressor protein p53 restricted by HLA-A2. These findings demonstrate functional TCR can be displayed on bacteriophage potentially leading to the development of novel applications involving TCR phage-display.

AU Weidanz J A; Card K F; Edwards A; Perlstein E; **Wong H C**

AB . . . showed the DO11.10 TCR-bearing phage could significantly inhibit stimulation of DO11.10 T hybridoma cells by competing for binding to immobilized MHC/peptide IA(d)/OVA(323-339). Flow cytometric analysis was carried out to evaluate direct binding of DO11.10 TCR-bearing phage onto the surface of cells. . .

L3 ANSWER 10 OF 12 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:533677 CAPLUS
DOCUMENT NUMBER: 127:204455
TITLE: Preparation and immunomodulatory activity of single-chain MHC mols.
INVENTOR(S): **Rhode, Peter R.; Jiao, Jin-An; Burkhardt, Martin; Wong, Hing C.**
PATENT ASSIGNEE(S): Dade International, Inc., USA; Rhode, Peter R.; Jiao, Jin-An; Burkhardt, Martin; Wong, Hing C.
SOURCE: PCT Int. Appl., 216 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9728191	A1	19970807	WO 1997-US1617	19970130
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
US 5869270	A	19990209	US 1996-596387	19960131
CA 2244755	AA	19970807	CA 1997-2244755	19970130
AU 9722538	A1	19970822	AU 1997-22538	19970130
AU 729672	B2	20010208		
EP 877760	A1	19981118	EP 1997-905709	19970130
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2000515363	T2	20001121	JP 1997-527863	19970130
US 6309645	B1	20011030	US 1998-67615	19980428
US 2002034513	A1	20020321	US 2001-848164	20010503

PRIORITY APPLN. INFO.: US 1996-596387 A 19960131
WO 1997-US1617 W 19970130
US 1998-67615 XX 19980428

AB The present invention relates to novel complexes of major histocompatibility complex (MHC) mols. and uses of such complexes. In one aspect, the invention relates to loaded MHC complexes that include at least one MHC mol. with a peptide-binding groove and a presenting peptide non-covalently linked to the MHC protein. In another aspect, the invention features single chain MHC class II peptide fusion complexes with a presenting peptide covalently linked to the peptide binding groove of the complex. MHC complexes are useful for a variety of applications including: (1) in vitro screens for identification and isolation of peptides that modulate activity of selected T cells, including peptides that are T cell receptor antagonists and partial agonists, and (2) methods for suppressing or inducing an immune response in a mammal.

TI Preparation and immunomodulatory activity of single-chain MHC mols.

IN **Rhode, Peter R.; Jiao, Jin-An; Burkhardt, Martin; Wong, Hing C.**

AB The present invention relates to novel complexes of major histocompatibility complex (MHC) mols. and uses of such complexes. In one aspect, the invention relates to loaded MHC complexes that include at least one MHC mol. with a peptide-binding groove and a presenting peptide non-covalently linked to the MHC protein. In another aspect, the invention features single chain MHC class II peptide fusion complexes with a presenting peptide covalently linked to the peptide binding groove of the complex. MHC complexes are useful for a variety of applications including: (1) in vitro screens for identification and isolation of peptides that modulate activity of selected T cells, including peptides that are T cell receptor antagonists and partial agonists, and (2) methods for suppressing or inducing an immune response in a mammal.

ST single chain MHC peptide immunomodulation

IT I-A antigen
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); PUR (Purification or recovery); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(I-As antigen, sol. single-chain; prepn. and immunomodulatory activity of single-chain MHC mols.)

IT Animal cell line
(NSO; immunomodulatory activity of single-chain MHC mols. expressed by)

IT Synthetic genes
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(animal; prepn. and immunomodulatory activity of single-chain MHC mols.)

IT Class I MHC antigens
RL: BAC (Biological activity or effector, except adverse); BPN

(Biosynthetic preparation); PUR (Purification or recovery); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(complexes with peptides; prepn. and immunomodulatory activity of single-chain MHC mols.)

IT Peptides, biological studies
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); PUR (Purification or recovery); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(complexes, with MHC antigens; prepn. and immunomodulatory activity of single-chain MHC mols.)

IT Class II MHC antigens
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); PUR (Purification or recovery); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(complexes, with peptides; prepn. and immunomodulatory activity of single-chain MHC mols.)

IT Class II MHC antigens
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); PUR (Purification or recovery); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(fusion products, single-chain, with peptides; prepn. and immunomodulatory activity of single-chain MHC mols.)

IT Peptides, biological studies
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); PUR (Purification or recovery); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(fusion products, with MHC antigens; prepn. and immunomodulatory activity of single-chain MHC mols.)

IT IgG2b
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); PUR (Purification or recovery); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(fusion products, with MHC class II; prepn. and immunomodulatory activity of single-chain MHC mols.)

IT Immunization
(genetic; prepn. and immunomodulatory activity of single-chain MHC mols.)

IT Allergic encephalomyelitis
Allergy inhibitors
Anergy
Antidiabetic agents
Antigen-presenting cell
Antirheumatic drugs
Autoimmune diseases
B cell (lymphocyte)
Dendritic cell
Helper T cell
Immunostimulants
Immunosuppressants
Immunotherapy
Multiple sclerosis
Myasthenia gravis
T cell activation
T cell proliferation
(immunomodulatory activity of single-chain MHC mols.)

IT TCR (T cell receptors)
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(immunomodulatory activity of single-chain MHC mols. in interaction with)

IT Immunodeficiency
(immunomodulatory activity of single-chain MHC mols. in relation to)

IT Molecular cloning
Plasmid vectors
(prepn. and immunomodulatory activity of single-chain MHC mols.)

IT Immunoscintigraphy
(prepn. and immunomodulatory activity of single-chain MHC mols. in relation to)

IT Class II MHC antigens
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); PUR (Purification or recovery); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(single-chain; prepn. and immunomodulatory activity of single-chain MHC mols.)

IT HLA-DR1 antigen
I-Ad antigen
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); PUR (Purification or recovery); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(sol. single-chain; prepn. and immunomodulatory activity of single-chain MHC mols.)

IT HLA-DP antigen
HLA-DQ antigen
I-E antigen
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(sol. single-chain; prepn. and immunomodulatory activity of single-chain MHC mols.)

IT Genes (animal)
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(synthetic; prepn. and immunomodulatory activity of single-chain MHC mols.)

IT 176670-35-2 176670-37-4 194674-96-9
RL: PRP (Properties)
(amino acid sequence; prepn. and immunomodulatory activity of single-chain MHC mols.)

IT 194549-26-3
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(as linker for single-chain MHC class II-peptide fusion mol.)

IT 194674-93-6 194674-94-7 194674-95-8
RL: PRP (Properties)
(nucleotide sequence; prepn. and immunomodulatory activity of single-chain MHC mols.)

L3 ANSWER 11 OF 12 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:302467 CAPLUS
DOCUMENT NUMBER: 124:340931
TITLE: Histocompatibility antigen MHC fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening
INVENTOR(S): **Wong, Hing C.; Rhode, Peter R.**; Weidanz, Jon A.; Grammer, Susan; Edwards, Ann C.; Chavaillaz, Pierre-Andre; **Jiao, Jin-An**
PATENT ASSIGNEE(S): Dade International, Inc., USA
SOURCE: PCT Int. Appl., 208 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9604314	A1	19960215	WO 1995-US9816	19950731
W: AU, CA, JP, US, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
CA 2196085	AA	19960215	CA 1995-2196085	19950731
AU 9534039	A1	19960304	AU 1995-34039	19950731
AU 696177	B2	19980903		
EP 776339	A1	19970604	EP 1995-930790	19950731
EP 776339	B1	20001011		
R: BE, CH, DE, DK, ES, FR, GB, IT, LI, LU, NL				
JP 10503379	T2	19980331	JP 1995-506744	19950731
EP 997477	A2	20000503	EP 1999-124343	19950731
R: BE, CH, DE, DK, ES, FR, GB, IT, LI, LU, NL				
ES 2152424	T3	20010201	ES 1995-930790	19950731

PRIORITY APPLN. INFO.: US 1994-283302 A2 19940729
US 1995-382454 A 19950201
EP 1995-930790 A3 19950731
WO 1995-US9816 W 19950731

AB The present invention relates to novel complexes of major histocompatibility complex (**MHC**) mols. and uses of such complexes. In particular, the invention relates to **MHC** fusion complexes that contain an **MHC** mol. with a peptide-binding groove and a presenting peptide covalently linked to the **MHC** protein. Fusion complexes of the invention are useful for a variety of applications including in vitro screens for identification and isolation of peptides that modulate activity of selected T cells, including peptides that are T cell receptor antagonists and partial agonists, methods of suppressing an immune response of a mammal and methods for inducing an immune response in a mammal.

TI Histocompatibility antigen **MHC** fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening

IN **Wong, Hing C.; Rhode, Peter R.**; Weidanz, Jon A.; Grammer, Susan; Edwards, Ann C.; Chavaillaz, Pierre-Andre; **Jiao, Jin-An**

AB The present invention relates to novel complexes of major histocompatibility complex (**MHC**) mols. and uses of such complexes. In particular, the invention relates to **MHC** fusion complexes that contain an **MHC** mol. with a peptide-binding groove and a presenting peptide covalently linked to the **MHC** protein. Fusion complexes of the invention are useful for a variety of applications including in vitro screens for identification and isolation of peptides that modulate activity of selected T cells, including peptides that are T cell receptor antagonists and partial agonists, methods of suppressing an immune response of a mammal and methods for inducing an immune response in a mammal.

ST antigen **MHC** fusion TcR receptor antagonist; immune response regulator fusion protein recombinant

IT Genetic element
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(Kozak sequence; histocompatibility antigen **MHC** fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening)

IT Peptides, biological studies
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(TcR antagonist; histocompatibility antigen **MHC** fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening)

IT Allergy
(chronic; histocompatibility antigen **MHC** fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening)

IT Animal cell
(expression host; histocompatibility antigen **MHC** fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening)

IT Genetic vectors
(expression; histocompatibility antigen **MHC** fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening)

IT Proteins, specific or class
RL: BUU (Biological use, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(gene B7, T-cell costimulatory factor; histocompatibility antigen **MHC** fusion products with T-cell receptor antagonist or other presenting peptide and use for immune response regulation and T-cell-modulator screening)

IT Proteins, specific or class
RL: BUU (Biological use, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(gene B7-2, T-cell costimulatory factor; histocompatibility antigen **MHC** fusion products with T-cell receptor antagonist or other presenting peptide and use for immune response regulation and T-cell-modulator screening)

IT Autoimmune disease
Immunostimulants
Immunosuppressants
Molecular cloning
Mouse
Multiple sclerosis
Myasthenia gravis
Protein sequences
Vaccines
(histocompatibility antigen MHC fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening)

IT Pharmaceutical dosage forms
(i.m.; histocompatibility antigen MHC fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening)

IT Pharmaceutical dosage forms
(intradermal; histocompatibility antigen MHC fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening)

IT Histocompatibility antigens
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(HLA-DR1, fusion products; histocompatibility antigen MHC fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening)

IT Histocompatibility antigens
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(MHC (major histocompatibility complex), fusion products; histocompatibility antigen MHC fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening)

IT Lymphocyte
(T-cell, histocompatibility antigen MHC fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening)

IT Antigen receptors
Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TCR (T-cell antigen receptor), TcR antagonist; histocompatibility antigen MHC fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening)

IT Gene, microbial
RL: BPR (Biological process); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)
(chimeric, histocompatibility antigen MHC fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening)

IT Deoxyribonucleic acid sequences
(complementary, histocompatibility antigen MHC fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening)

IT Immunoglobulins
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(fusion products, histocompatibility antigen MHC fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening)

IT Diabetes mellitus
(insulin-dependent, histocompatibility antigen MHC fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening)

IT Pharmaceutical dosage forms
(oral, histocompatibility antigen MHC fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening)

IT Arthritis
(rheumatoid, histocompatibility antigen MHC fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening)

IT Pharmaceutical dosage forms
(transdermal, histocompatibility antigen MHC fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening)

IT 176670-35-2P 176670-37-4P 176670-39-6P
RL: BPN (Biosynthetic preparation); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(amino acid sequence; histocompatibility antigen MHC fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening)

IT 176708-18-2 176708-23-9
RL: PRP (Properties)
(amino acid sequence; histocompatibility antigen MHC fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening)

IT 98037-54-8, Nuclease, restriction endodeoxyribo-, AflII
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(cleavage site; histocompatibility antigen MHC fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening)

IT 176670-34-1 176670-36-3 176670-38-5
RL: BPR (Biological process); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process); USES (Uses)
(nucleotide sequence; histocompatibility antigen MHC fusion products with T-cell receptor antagonist or other presenting peptide,

recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening)
IT 176670-32-9 176670-33-0
RL: PRP (Properties)
(nucleotide sequence; histocompatibility antigen MHC fusion products with T-cell receptor antagonist or other presenting peptide, recombinant fusion protein, and use for immune response regulation and T-cell-modulator screening)

L3 ANSWER 12 OF 12 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 97098715 MEDLINE
DOCUMENT NUMBER: 97098715 PubMed ID: 8943392
TITLE: Single-chain MHC class II molecules induce T cell activation and apoptosis.
AUTHOR: **Rhode P R; Burkhardt M; Jiao J**
; Siddiqui A H; Huang G P; **Wong H C**
CORPORATE SOURCE: Sunol Molecular Corporation, Miami, FL 33172, USA.
SOURCE: JOURNAL OF IMMUNOLOGY, (1996 Dec 1) 157 (11) 4885-91.
Journal code: IFB; 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19961227
AB MHC class II/peptide complexes displayed on the surface of APCs play a pivotal role in initiating specific T cell responses. Evidence is presented here that components of this heterotrimeric complex can be genetically linked into a single polypeptide chain. Soluble single-chain (sc) murine class II IA(d) molecules with and without covalently attached peptides were produced in a recombinant baculovirus-insect cell expression system. Correct conformation of these molecules was verified based on 1) reactivity to Abs directed against conformational epitopes in IA(d) and 2) peptide-specific recognition of the IA(d)/peptide complexes by T cells. Both sc class II molecules loaded the appropriate peptides and sc class II/peptide fusions were effective in stimulating T cell responses, including cytokine release and apoptosis. Mammalian cells were also found to be capable of expressing functional sc class II molecules on their cell surfaces. The findings reported here open up the possibility of producing large amounts of stable sc class II/peptide fusion molecules for structural characterization and immunotherapeutic applications.
TI Single-chain MHC class II molecules induce T cell activation and apoptosis.
AU **Rhode P R; Burkhardt M; Jiao J**; Siddiqui A H; Huang G P; **Wong H C**
AB MHC class II/peptide complexes displayed on the surface of APCs play a pivotal role in initiating specific T cell responses. Evidence. .

1-31-96 pm only

=> s (single (1N) chain) (10N) (class (1N) II)
L4 62 (SINGLE (1N) CHAIN) (10N) (CLASS (1N) II)

=> dup rem l4
PROCESSING COMPLETED FOR L4
L5 30 DUP REM L4 (32 DUPLICATES REMOVED)

=> s l5 and PD<19960131
'19960131' NOT A VALID FIELD CODE
3 FILES SEARCHED...
L6 2 L5 AND PD<19960131

=> dis l6 1-2 ibib abs kwic

L6 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1986:419749 CAPLUS
DOCUMENT NUMBER: 105:19749
TITLE: HLA-DR .alpha. chain expression in human thyroid cells
AUTHOR(S): Piccinini, Linda A.; Schachter, Beth S.; Davies, Terry F.
CORPORATE SOURCE: Dep. Med., Mount Sinai Sch. Med., New York, NY, 10029, USA
SOURCE: Endocrinology (Baltimore) (1986), 118(6), 2611-13
CODEN: ENDOAO; ISSN: 0013-7227
DOCUMENT TYPE: Journal
LANGUAGE: English
AB By using a cDNA probe encoding the human major histocompatibility **class II** antigen HLA-DR .alpha.-**chain**, a **single** DR .alpha.-chain-specific transcript was detected in total cellular RNA prepd. from human thyroid tissue. The hybridizable RNA in thyroid samples was indistinguishable in size from the DR mRNA in the Raji human B lymphoblastoid cell line. Of the thyroid glands examd., samples from patients with autoimmune thyroid disease consistently demonstrated the highest DR .alpha.-chain transcript levels, with a mean of .apprx.62% of the levels found in DR-pos. Raji cells. Cytoplasmic dot-blot analyses of 5-day thyroid cell cultures depleted of lymphocytes and monocytes indicated that normal thyrocytes contain readily detectable levels of DR .alpha.-chain mRNA. These transcript levels varied, with a mean of .apprx.16% relative to Raji cell control values, and were shown to correlate after lectin or gamma interferon stimulation with enhanced nos. of immunoreactive DR antigen-pos. cells. Such findings demonstrate expression of HLA class II antigen genes in normal, unstimulated human thyroid cells and suggest that quant. variation in thyroid class II antigen (DR) gene expression may be a major factor in thyroid immunoregulation.
SO Endocrinology (Baltimore) (1986), 118(6), 2611-13
CODEN: ENDOAO; ISSN: 0013-7227
AB By using a cDNA probe encoding the human major histocompatibility **class II** antigen HLA-DR .alpha.-**chain**, a **single** DR .alpha.-chain-specific transcript was detected in total cellular RNA prepd. from human thyroid tissue. The hybridizable RNA in thyroid samples was indistinguishable in size from the DR mRNA in the Raji human B lymphoblastoid cell line. Of the thyroid glands examd., samples from patients with autoimmune thyroid disease consistently demonstrated the highest DR .alpha.-chain transcript levels, with a mean of .apprx.62% of the levels found in DR-pos. Raji cells. Cytoplasmic dot-blot analyses of 5-day thyroid cell cultures depleted of lymphocytes and monocytes indicated that normal thyrocytes contain readily detectable levels of DR .alpha.-chain mRNA. These transcript levels varied, with a mean of .apprx.16% relative to Raji cell control values, and were shown to

correlate after lectin or gamma interferon stimulation with enhanced nos. of immunoreactive DR antigen-pos. cells. Such findings demonstrate expression of HLA class II antigen genes in normal, unstimulated human thyroid cells and suggest that quant. variation in thyroid class II antigen (DR) gene expression may be a major factor in thyroid immunoregulation.

L6 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1978:418588 CAPLUS
DOCUMENT NUMBER: 89:18588
TITLE: Cytochrome-C and copper protein evolution in prokaryotes
AUTHOR(S): Ambler, R. P.
CORPORATE SOURCE: Dep. Mol. Biol., Edinburgh, Scot.
SOURCE: Evol. Metalloenzymes, Metalloproteins Relat. Mater., Proc. Symp. (1977), 100-18. Editor(s): Leigh, G. J. Sci. Reviews Ltd.: London, Engl.
CODEN: 37XGAL
DOCUMENT TYPE: Conference
LANGUAGE: English
AB An amino acid sequence-based classification for cytochromes c is proposed having as its basis the location of the heme-binding sequence in the polypeptide sequence; e.g., class I cytochromes comprise those proteins where the heme group is attached near the N-terminus of a **single** polypeptide **chain** of 80-120 residues and **class** II includes those cytochromes c where the heme group is attached near the C-terminus of a single polypeptide chain of .apprx.120 residues. The relation of the structure of the cytochromes c and of the electron-transport blue Cu proteins, the azurins and the plastocyanins, to their evolution in prokaryotes is discussed.
SO Evol. Metalloenzymes, Metalloproteins Relat. Mater., Proc. Symp. (1977), 100-18. Editor(s): Leigh, G. J. Publisher: Sci. Reviews Ltd., London, Engl.
CODEN: 37XGAL
AB An amino acid sequence-based classification for cytochromes c is proposed having as its basis the location of the heme-binding sequence in the polypeptide sequence; e.g., class I cytochromes comprise those proteins where the heme group is attached near the N-terminus of a **single** polypeptide **chain** of 80-120 residues and **class** II includes those cytochromes c where the heme group is attached near the C-terminus of a single polypeptide chain of .apprx.120 residues. The relation of the structure of the cytochromes c and of the electron-transport blue Cu proteins, the azurins and the plastocyanins, to their evolution in prokaryotes is discussed.

=> dis his

(FILE 'HOME' ENTERED AT 15:26:37 ON 04 MAY 2002)

FILE 'MEDLINE, CAPLUS, EMBASE, BIOSIS' ENTERED AT 15:26:47 ON 04 MAY 2002
L1 4867 S RHODE P?/AU OR JIAO J?/AU OR BURKHARDT M?/AU OR WONG H?/AU
L2 16 S L1 AND MHC
L3 12 DUP REM L2 (4 DUPLICATES REMOVED)
L4 62 S (SINGLE (1N) CHAIN) (10N) (CLASS (1N) II)
L5 30 DUP REM L4 (32 DUPLICATES REMOVED)
L6 2 S L5 AND PD<19960131

=> s l5 not l6
L7 28 L5 NOT L6

=> dis l7 1-28 ibib abs

L7 ANSWER 1 OF 28 MEDLINE
ACCESSION NUMBER: 2002130991 MEDLINE
DOCUMENT NUMBER: 21848278 PubMed ID: 11859151
TITLE: A **single-chain class** II MHC-IgG3 fusion protein inhibits autoimmune arthritis by induction of antigen-specific hyporesponsiveness.
AUTHOR: Zuo Li; Cullen Constance M; DeLay Monica L; Thornton Sherry; Myers Linda K; Rosloniec Edward F; Boivin Gregory P; Hirsch Raphael
CORPORATE SOURCE: Division of Rheumatology, Children's Hospital Medical Center, University of Cincinnati, 3333 Burnet Avenue, Cincinnati, OH 45229, USA.
CONTRACT NUMBER: AI 34958 (NIAID)
AR 47363 (NIAMS)
SOURCE: JOURNAL OF IMMUNOLOGY, (2002 Mar 1) 168 (5) 2554-9.
Journal code: 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200203
ENTRY DATE: Entered STN: 20020228
Last Updated on STN: 20020317
Entered Medline: 20020315
AB T cells play a central role in many autoimmune diseases. A method to specifically target the function of autoreactive T cell clones would avoid the global immunosuppression associated with current therapies. To develop a molecule capable of inhibiting autoreactive T cell responses in vivo, single-chain peptide-I-A-IgG3 fusion proteins were constructed and expressed in both mammalian and insect cells. The fusion proteins were designed with an IgG3 Fc moiety to make them divalent, allowing TCR cross-linking, while lacking FcR binding and costimulation. The fusion proteins stimulated T cell hybridomas in vitro in a peptide-specific, MHC-restricted manner but failed to do so in soluble form. In vivo administration of an I-A(q) fusion protein, containing an immunodominant collagen II peptide, significantly delayed the onset and reduced the severity of collagen-induced arthritis in DBA/1 mice by induction of Ag-specific hyporesponsiveness. Such fusion proteins may be useful to study novel therapeutic approaches for T cell-mediated autoimmune diseases.

L7 ANSWER 2 OF 28 MEDLINE
ACCESSION NUMBER: 2001086806 MEDLINE
DOCUMENT NUMBER: 20558264 PubMed ID: 11106438
TITLE: Expression and characterization of truncated forms of humanized L243 IgG1. Architectural features can influence synthesis of its oligosaccharide chains and affect superoxide production triggered through human Fcgamma receptor I.

AUTHOR: Lund J; Takahashi N; Popplewell A; Goodall M; Pound J D; Tyler R; King D J; Jefferis R
CORPORATE SOURCE: Department of Immunology, The Medical School, Birmingham, UK.. J.Lund@bham.ac.uk
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (2000 Dec) 267 (24) 7246-57.
Journal code: EMZ. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200101
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010118

AB The properties of IgG and its subcomponents are being exploited to generate new therapeutics with selected biological activities. In this study, a series of truncated, humanized IgG1 antibodies was expressed in Chinese hamster ovary cells, to evaluate the contribution of structural components to glycosylation and function. The series includes L243 IgG1 (alpha-MHC **Class** II) lacking a CH3 domain pair (DeltaCH3-IgG1), **single-chain** Fv fusion proteins with Fc or a hinge-CH2 domain, Fc with/out a hinge, and a single CH2 domain. Glycosylation of IgG Fc is important for recognition by effector ligands such as Fcgamma receptors. HPLC analysis of released and pyridylaminated oligosaccharides indicates that intact IgG1 and scFvFc antibodies are galactosylated and sialylated to levels similar to those observed previously for normal human IgG1. The truncated forms express increased levels of digalactosylated (30-83%) or sialylated (9-21%) oligosaccharide chains with the highest levels observed for the single CH2 domain. These data show which architectural components influence IgG glycosylation processing and that the (CH3)2 pair is particularly influential. When MHC Class II bearing (JY) cells were sensitized with L243 DeltaCH3-IgG1, scFvFc, or scFvhCH2 they elicited superoxide production, from U937 cells, at levels of 35-45% relative to that obtained for intact L243 IgG1 (100%). Mild reduction and alkylation of the hinge disulphide bonds of scFvhCH2 greatly decreased its capacity to trigger superoxide production. Thus, the L243 scFvhCH2 homo-dimer constitutes the minimal truncated form that binds the MHC Class II antigen and triggers superoxide production through FcgammaRI.

L7 ANSWER 3 OF 28 MEDLINE
ACCESSION NUMBER: 2001060697 MEDLINE
DOCUMENT NUMBER: 20536497 PubMed ID: 10965044
TITLE: Modulation of the peptide-binding specificity of a **single-chain class II** major histocompatibility complex.
AUTHOR: Kim S T; Byun S M
CORPORATE SOURCE: Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), Kusung-dong, Yusung-gu, Taejon 305-701, Korea.. smbyun@mail.kaist.ac.kr
SOURCE: JOURNAL OF BIOCHEMISTRY, (2000 Sep) 128 (3) 449-54.
Journal code: HIF. ISSN: 0021-924X.
PUB. COUNTRY: Japan
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200012
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001222

AB We designed and expressed a **single-chain class** II major histocompatibility complex molecule capable of forming a stable complex with an antigenic peptide. The peptide-binding preference of the single-chain (sc) human leukocyte antigen derived from DRB5(*)0101 (DR51) was determined to be similar to that of the authentic one, which requires a bulky hydrophobic residue at position-1 (P1) as a primary anchor. For modulation of the peptide-binding affinity, we modified binding pocket 1 of sc DR51 by site-directed mutagenesis. The relative binding affinity of the engineered sc DR51 for several P1-substituted peptides was measured by competition assaying with a fluorescence labeled peptide. The sc DR51 molecule showed high affinity to the self-peptide derived from myelin basic protein, 87-98 with Phe as the P1 residue (F90F). While reduction of pocket 1 volume (betaG86V) decreased the affinity of F90F, it rather increased the affinity of the Ala-substituted peptide as to the P1 residue (F90A). Through more extensive engineering in the peptide-binding groove of the sc DR51 molecule, it is expected that we can construct sc DR51 variants with various peptide ligand motifs.

L7 ANSWER 4 OF 28 MEDLINE
ACCESSION NUMBER: 1999287109 MEDLINE
DOCUMENT NUMBER: 99287109 PubMed ID: 10360364
TITLE: Structure, specificity and CDR mobility of a **class** II restricted **single-chain** T-cell receptor.
AUTHOR: Hare B J; Wyss D F; Osburne M S; Kern P S; Reinherz E L; Wagner G
CORPORATE SOURCE: Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115, USA.
SOURCE: NATURE STRUCTURAL BIOLOGY, (1999 Jun) 6 (6) 574-81.
Journal code: B98; 9421566. ISSN: 1072-8368.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1BWM
ENTRY MONTH: 199906
ENTRY DATE: Entered STN: 19990712
Last Updated on STN: 19990712
Entered Medline: 19990623

AB Using NMR spectroscopy, we determined the solution structure of a single-chain T-cell receptor (scTCR) derived from the major histocompatibility complex (MHC) class II-restricted D10 TCR. The conformations of complementarity-determining regions (CDRs) 3beta and 1alpha and surface properties of 2alpha are different from those of related class I-restricted TCRs. We infer a conserved orientation for TCR V(alpha) domains in complexes with both class I and II MHC-peptide ligands, which implies that small structural variations in V(alpha) confer MHC class preference. High mobility of CDR3 residues relative to other CDR or framework residues (picosecond time scale) provides insight into immune recognition and selection mechanisms.

L7 ANSWER 5 OF 28 MEDLINE
ACCESSION NUMBER: 1999049826 MEDLINE
DOCUMENT NUMBER: 99049826 PubMed ID: 9834080
TITLE: Two-domain MHC class II molecules form stable complexes with myelin basic protein 69-89 peptide that detect and inhibit rat encephalitogenic T cells and treat experimental autoimmune encephalomyelitis.
AUTHOR: Burrows G G; Bebo B F Jr; Adlard K-L; Vandenbark A A; Offner H
CORPORATE SOURCE: Veterans Affairs Medical Center, Department of Neurology, Oregon Health Sciences University, Portland 97201, USA.. ggb@ohsu.edu
SOURCE: JOURNAL OF IMMUNOLOGY, (1998 Dec 1) 161 (11) 5987-96. Journal code: IFB; 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199812
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 20000303
Entered Medline: 19981221

AB We designed and expressed in bacteria a **single-chain** two-domain MHC **class** II molecule capable of binding and forming stable complexes with antigenic peptide. The prototype "betalalpha1" molecule included the beta1 domain of the rat RT1.B class II molecule covalently linked to the amino terminus of the alpha1 domain. In association with the encephalitogenic myelin basic protein (MBP) 69-89 peptide recognized by Lewis rat T cells, the betalalpha1/MBP-69-89 complex specifically labeled and inhibited activation of MBP-69-89 reactive T cells in an IL-2-reversible manner. Moreover, this complex both suppressed and treated clinical signs of experimental autoimmune encephalomyelitis and inhibited delayed-type hypersensitivity reactions and lymphocyte proliferation in an Ag-specific manner. These data indicate that the betalalpha1/MBP-69-89 complex functions as a simplified natural TCR ligand with potent inhibitory activity that does not require additional signaling from the beta2 and alpha2 domains. This new class of small soluble polypeptide may provide a template for designing human homologues useful in detecting and regulating potentially autopathogenic T cells.

L7 ANSWER 6 OF 28 MEDLINE
ACCESSION NUMBER: 97439473 MEDLINE
DOCUMENT NUMBER: 97439473 PubMed ID: 9295029
TITLE: A recombinant **single-chain** human **class** II MHC molecule (HLA-DR1) as a covalently linked heterotrimer of alpha chain, beta chain, and antigenic peptide, with immunogenicity in vitro and reduced affinity for bacterial superantigens.
AUTHOR: Zhu X; Bavari S; Ulrich R; Sadegh-Nasseri S; Ferrone S; McHugh L; Mage M
CORPORATE SOURCE: Laboratory of Biochemistry, DCBDC, NCI, NIH, Bethesda, MD 20892, USA.
SOURCE: EUROPEAN JOURNAL OF IMMUNOLOGY, (1997 Aug) 27 (8) 1933-41. Journal code: EN5; 1273201. ISSN: 0014-2980.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199709
ENTRY DATE: Entered STN: 19971013
Last Updated on STN: 19971013
Entered Medline: 19970930

AB Major histocompatibility complex (MHC) class II molecules bind to numerous peptides and display these on the cell surface for T cell recognition. In a given immune response, receptors on T cells recognize antigenic peptides that are a minor population of MHC class II-bound peptides. To control which peptides are presented to T cells, it may be desirable to use recombinant MHC molecules with covalently bound antigenic peptides. To study T cell responses to such homogeneous peptide-MHC complexes, we engineered an HLA-DR1 cDNA coding for influenza hemagglutinin, influenza matrix, or HIV p24 gag peptides covalently attached via a peptide spacer to the N terminus of the DR1 beta chain. Co-transfection with DR alpha cDNA into mouse L cells resulted in surface expression of HLA-DR1 molecules that reacted with monoclonal antibodies (mAb) specific for correctly folded HLA-DR epitopes. This suggested that the spacer and peptide did not alter expression or folding of the molecule. We then engineered an additional peptide spacer between the C terminus of a truncated beta chain (without transmembrane or cytoplasmic domains) and the N terminus of full-length DR alpha chain. Transfection of this cDNA into mouse L cells resulted in surface expression of the entire covalently linked heterotrimer of peptide, beta chain, and alpha chain with the expected molecular mass of approximately 66 kDa. These single-chain HLA-DR1 molecules reacted with mAb specific for correctly folded HLA-DR epitopes, and identified one mAb with [MHC + peptide] specificity. Affinity-purified soluble secreted **single-chain** molecules with truncated alpha chain moved in electrophoresis as compact **class** II MHC dimers. Cell surface two-**chain** or **single-chain** HLA-DR1 molecules with a covalent HA peptide stimulated HLA-DR1-restricted HA-specific T cells. They were immunogenic in vitro for peripheral blood mononuclear cells. The two-chain and single-chain HLA-DR1 molecules with covalent HA peptide had reduced binding for the bacterial superantigens staphylococcal enterotoxin A and B and almost no binding for toxic shock syndrome toxin-1. The unique properties of these engineered HLA-DR1 molecules may facilitate our understanding of the complex nature of antigen recognition and aid in the development of novel vaccines with reduced superantigen binding.

L7 ANSWER 7 OF 28 MEDLINE
ACCESSION NUMBER: 97098715 MEDLINE
DOCUMENT NUMBER: 97098715 PubMed ID: 8943392
TITLE: **Single-chain** MHC **class** II molecules induce T cell activation and apoptosis.
AUTHOR: Rhode P R; Burkhardt M; Jiao J; Siddiqui A H; Huang G P; Wong H C
CORPORATE SOURCE: Sunol Molecular Corporation, Miami, FL 33172, USA.
SOURCE: JOURNAL OF IMMUNOLOGY, (1996 Dec 1) 157 (11) 4885-91. Journal code: IFB; 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199612

applicb

ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19961227

AB MHC class II/peptide complexes displayed on the surface of APCs play a pivotal role in initiating specific T cell responses. Evidence is presented here that components of this heterotrimeric complex can be genetically linked into a **single** polypeptide **chain**. Soluble **single-chain** (sc) murine **class** II IA(d) molecules with and without covalently attached peptides were produced in a recombinant baculovirus-insect cell expression system. Correct conformation of these molecules was verified based on 1) reactivity to Abs directed against conformational epitopes in IA(d) and 2) peptide-specific recognition of the IA(d)/peptide complexes by T cells. Both sc class II molecules loaded the appropriate peptides and sc class II/peptide fusions were effective in stimulating T cell responses, including cytokine release and apoptosis. Mammalian cells were also found to be capable of expressing functional sc class II molecules on their cell surfaces. The findings reported here open up the possibility of producing large amounts of stable sc class II/peptide fusion molecules for structural characterization and immunotherapeutic applications.

L7 ANSWER 8 OF 28 MEDLINE
ACCESSION NUMBER: 96406432 MEDLINE
DOCUMENT NUMBER: 96406432 PubMed ID: 8810565
TITLE: HLA class II alleles and leprosy (Hansen's disease) classified by WHO-MDT criteria.
AUTHOR: Joko S; Numaga J; Masuda K; Namisato M; Maeda H
CORPORATE SOURCE: Department of Ophthalmology, University of Tokyo School of Medicine, Japan.
SOURCE: NIPPON RAI GAKKAI ZASSHI. JAPANESE JOURNAL OF LEPROSY, (1996 Jul) 65 (2) 121-7.
Journal code: HDT; 7901165. ISSN: 0386-3980.
PUB. COUNTRY: Japan
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Japanese
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19961204

AB Human leukocyte antigens (HLA) class II alleles were analyzed in Japanese leprosy patients to ascertain whether immunogenetic differences exist among the forms of leprosy in classification of World Health Organization-recommended multidrug therapy (WHO-MDT). The subjects were 86 unrelated Japanese leprosy patients, including 62 multibacillary leprosy (MBL), 24 paucibacillary leprosy (PBL). Controls were 114 unrelated healthy subjects. Genotyping of HLA **class** II alleles was performed by using the polymerase **chain** reaction-**single** strand conformation polymorphism (PCR-SSCP) and PCR-restriction fragment length polymorphism (RFLP) methods. The frequencies of HLA-DRB1* 1501, * 1502 and DRB5* 0101, * 0102 and DQA1* 0102 and DQB1* 0602 were significantly increased in the whole patients (44.2%, 34.9%, 44.2%, 34.9%, 53.4% and 41.9%, respectively) as compared with the control subjects (14.0%, 21.1%, 14.0%, 21.1%, 27.2% and 13.2%, respectively). On the other hand, the frequencies of HLA-DRB1* 0405, * 0803, * 0901 and DQA1* 03 and DQB1* 0401 were significantly decreased in the whole patients (10.5%, 5.8%, 16.3%, 41.9% and 9.3%, respectively) as compared with the control subjects (29.8%, 17.5%, 30.7%, 78.1% and 29.8%, respectively). When MBL and PBL patients were compared, the frequencies of HLA-DRB1* 1501, DRB5* 0101 and DQB1* 0602 were significantly increased in the MBL patients (51.6%, 51.6% and 48.4%, respectively) as compared with the PBL patients (25.0%, respectively). Our results suggest that HLA-DRB1* 1501, DRB5* 0101 and DQB1* 0602 contribute to the susceptibility to the Japanese MBL.

L7 ANSWER 9 OF 28 MEDLINE
ACCESSION NUMBER: 95187965 MEDLINE
DOCUMENT NUMBER: 95187965 PubMed ID: 7882148
TITLE: Graft rejection across transgene-encoded MHC class II molecules.
AUTHOR: Rosay P; Hergueux J; Benoist C; Mathis D
CORPORATE SOURCE: Laboratoire de Genetique Moleculaire des Eucaryotes du CNRS et INSERM U. 184, Institut de Chimie Biologique, Strasbourg, France.
CONTRACT NUMBER: RO1-AI26636-01 (NIAID)
SOURCE: COMPTES RENDUS DE L ACADEMIE DES SCIENCES. SERIE III, SCIENCES DE LA VIE, (1994 Jul) 317 (7) 639-43.
Journal code: CA1; 8503078. ISSN: 0764-4469.
PUB. COUNTRY: France
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199504
ENTRY DATE: Entered STN: 19950425
Last Updated on STN: 19950425
Entered Medline: 19950411

AB To investigate the capacity of class II gene products of the major histocompatibility complex to serve as targets for allograft rejection, we have used lines of transgenic mice which express such genes on a common genetic background. These lines allow us to test the function of **single** **class** II molecules, or of **single** **chains** of the **class** II heterodimers, in graft rejection or tolerance induction. Our data show that some class II molecules (A alpha, A beta) can induce very efficient rejection, while others are relatively inert (E), and that tolerance induction requires matching for both chains of the target class II heterodimers.

L7 ANSWER 10 OF 28 MEDLINE
ACCESSION NUMBER: 95173602 MEDLINE
DOCUMENT NUMBER: 95173602 PubMed ID: 7532684
TITLE: The T cell response of HLA-DR transgenic mice to human myelin basic protein and other antigens in the presence and absence of human CD4.
AUTHOR: Altmann D M; Douek D C; Frater A J; Hetherington C M; Inoko H; Elliott J I
CORPORATE SOURCE: MRC Clinical Sciences Centre, Royal Postgraduate Medical School, Hammersmith Hospital, London, United Kingdom.
SOURCE: JOURNAL OF EXPERIMENTAL MEDICINE, (1995 Mar 1) 181 (3) 867-75.
Journal code: I2V; 2985109R. ISSN: 0022-1007.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 199503
ENTRY DATE: Entered STN: 19950407
Last Updated on STN: 19960129
Entered Medline: 19950324

AB Analysis of HLA **class** II transgenic mice has progressed in recent years from analysis of **single chain** HLA **class** II transgenes with expression of mixed mouse/human heterodimers to double transgenic mice expressing normal human heterodimers. Previous studies have used either HLA transgenic mice in which there is a species-matched interaction with CD4 or mice which lack this interaction. Since both systems are reported to generate HLA-restricted responses, the matter of the requirement for species-matched CD4 remains unclear. We have generated triple transgenic mice expressing three human transgenes, DRA, DRB, and CD4, and compared HLA-restricted responses to peptide between human-CD4+ (Hu-CD4+) and Hu-CD4- littermates. We saw no difference between Hu-CD4+ and Hu-CD4- groups, supporting the notion that for some responses at least the requirement for species-matched CD4 may not be absolute. Evidence for positive selection of mouse T cell receptors in HLA-DR transgenic mice came both from the acquisition of new, HLA-restricted responses to various peptides and from an increased frequency of T cells using the TCR V beta 4 gene segment. An important goal with respect to the analysis of function in HLA transgenic mice is the clarification of mechanisms which underpin the recognition of self-antigens in human autoimmune disease. As a first step towards 'humanized' disease models in HLA transgenic mice, we analyzed the responses of HLA-DR transgenic mice to the human MPB 139-154 peptide which has been implicated as an epitope recognized by T cells of multiple sclerosis patients. We obtained T cell responses to this epitope in transgenic mice but not in nontransgenic controls. This study suggests that HLA transgenic mice will be valuable in the analysis of HLA-restricted T cell epitopes implicated in human disease and possibly in the design of new disease models.

L7 ANSWER 11 OF 28 MEDLINE
ACCESSION NUMBER: 95035006 MEDLINE
DOCUMENT NUMBER: 95035006 PubMed ID: 7947938
TITLE: The interaction of cytochrome c and the heme domain of cytochrome P-450BM-3 with the reductase domain of cytochrome P-450BM-3.
AUTHOR: Klein M L; Fulco A J
CORPORATE SOURCE: Department of Biological Chemistry, UCLA School of Medicine.
CONTRACT NUMBER: GM23913 (NIGMS)
HL-07386 (NHLBI)
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA, (1994 Nov 11) 1201 (2) 245-50.
Journal code: A0W; 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199412
ENTRY DATE: Entered STN: 19950110
Last Updated on STN: 19980206
Entered Medline: 19941222

AB Cytochrome P-450BM-3 from Bacillus megaterium is a soluble, catalytically self-sufficient fatty acid mono-oxygenase that resembles the **Class** II P-450 systems of the eukaryotic endoplasmic reticulum. Its **single** polypeptide **chain** contains both a P-450 heme domain and an NADPH:P-450 reductase domain, each of which bears significant structural and functional homology with its microsomal counterparts. We report here that cytochrome c, which can accept NADPH-derived electrons from the reductase domain of P-450-BM-3, did not inhibit myristate hydroxylation catalyzed by P-450BM-3 or by two reductase domain mutant enzymes (W574Y, W574P) which have diminished hydroxylase activity relative to wild-type enzyme but retain cytochrome c reductase activity levels comparable to wild-type enzyme. Because reduced cytochrome c generated independently of the reductase domain of P-450BM-3 did not support myristate hydroxylation, it seems likely that cytochrome c binds to a site on the reductase domain which does not overlap the site of the heme domain interaction. We also found that myristate did not inhibit P-450BM-3-mediated cytochrome c reduction. Since neither substrate inhibited the conversion of the other, we conclude that the rate-limiting steps for both myristate hydroxylation and cytochrome c reduction by P-450BM-3 do not involve electron transfer through the reductase domain.

L7 ANSWER 12 OF 28 MEDLINE
ACCESSION NUMBER: 93216707 MEDLINE
DOCUMENT NUMBER: 93216707 PubMed ID: 8463285
TITLE: Critical residues involved in FMN binding and catalytic activity in cytochrome P450BM-3.
AUTHOR: Klein M L; Fulco A J
CORPORATE SOURCE: Department of Biological Chemistry, School of Medicine, University of California, Los Angeles 90024-1737.
CONTRACT NUMBER: GM23913 (NIGMS)
HL-07386 (NHLBI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 Apr 5) 268 (10) 7553-61.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199305
ENTRY DATE: Entered STN: 19930521
Last Updated on STN: 19930521
Entered Medline: 19930505

AB Cytochrome P450BM-3 from Bacillus megaterium is a soluble, catalytically self-sufficient fatty acid mono-oxygenase that, in structural organization and amino acid sequence, resembles the **Class II** (microsomal) P450 systems. Its **single** polypeptide **chain** contains both a P450 heme domain and an NADPH:P450 reductase domain, each of which bears significant homology with its microsomal counterparts. We report here the critical nature of three amino acids in the reductase domain of this enzyme with respect to FMN binding and catalytic activity. We used site-directed mutagenesis to change glycine 570 to bulkier amino acids; none of these mutant enzymes contained FMN after purification. We also made substitutions for tryptophan 574 and tyrosine 536, which by sequence analogy (Porter, T. D. (1991) Trends Biochem. Sci. 16, 154-158) were proposed to bind FMN through stacking of the aromatic rings with the isoalloxazine ring of the flavin. Mutants of tryptophan 574 which retained the aromatic side chain contained no less than 0.85 mol of FMN per mol of

enzyme, while aspartate and glycine substitutions yielded enzymes which did not incorporate FMN. Substitution of tyrosine 536 with aspartate gave an enzyme which contained 0.44 mol of FMN per mol of enzyme but was inactive as a fatty acid hydroxylase and had only 2% of wild-type cytochrome c reductase activity, while the glycine mutant at this position bound no FMN. Furthermore, although all of the mutant enzymes contained 1 mol of FAD per mol of enzyme, the Y536D mutant and those entirely lacking FMN retained no more than 40% of wild-type ferricyanide reductase activity. By assaying these enzymes in the presence of added FMN, we were able to assess the relative importance of the residues in the wild-type sequence with respect to their contribution to FMN binding. In addition, the aromatic mutants of tryptophan 574, which were nearly as active in cytochrome c reduction as wild-type P450BM-3, were only 20% as active in myristate hydroxylation as the wild-type enzyme, suggesting that this amino acid plays an important role in the flow of electrons between the P450 heme and reductase domains.

L7 ANSWER 13 OF 28 MEDLINE
ACCESSION NUMBER: 93056563 MEDLINE
DOCUMENT NUMBER: 93056563 PubMed ID: 1385534
TITLE: Preparative-scale purification and characterization of MHC class II monomers.
AUTHOR: Passmore D; Kopa D; Nag B
CORPORATE SOURCE: Anergen Inc., Redwood City, CA 94063.
SOURCE: JOURNAL OF IMMUNOLOGICAL METHODS, (1992 Nov 5) 155 (2) 193-200.
Journal code: IFE; 1305440. ISSN: 0022-1759.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199212
ENTRY DATE: Entered STN: 19930122
Last Updated on STN: 19960129
Entered Medline: 19921209

AB The MHC class II molecule is a heterodimeric glycoprotein consisting of one alpha and one beta polypeptide chain of almost identical molecular size. Recently it has been shown by others, and confirmed in our laboratory, that isolated monomers of murine MHC II molecules are capable of binding antigenic peptides like the alpha/beta intact heterodimer. In addition, preliminary results from our laboratory indicate that isolated **single chain**-peptide complexes of murine MHC **class** II molecules are capable of stimulating cloned T cells in an antigen specific manner. These results prompted us to isolate relatively large quantities of individual alpha and beta subunits of MHC II molecules for further in vitro and in vivo studies. Isolation of alpha and beta monomers proved to be difficult using conventional chromatographic methods. In this report we describe micro-preparative and preparative continuous flow electrophoresis methods by which milligram quantities of MHC II subunits can be purified. An optimal condition for the dissociation of heterodimeric MHC II into alpha and beta monomers was identified, and separation of human HLA DR2 and murine IAs monomers was accomplished. Both methods offer the resolving power of gel electrophoresis with the convenience of continuous sample elution. Purified MHC II subunits obtained by these methods were tested for their ability to bind antigenic peptides. Results presented in this study indicate that monomeric subunits of both human HLA-DR2 and murine IAs are equally active in specific binding of antigenic peptides like the native heterodimer.

L7 ANSWER 14 OF 28 MEDLINE
ACCESSION NUMBER: 88014331 MEDLINE
DOCUMENT NUMBER: 88014331 PubMed ID: 3309687
TITLE: Supplementary characteristics of anti-MHC class II monoclonal antibodies elicited by an ALL cell line: immunofluorescence cytofluorometry, C-dependent cytotoxicity, two-dimensional analysis of antigen.
AUTHOR: Chorvath B; Duraj J; Sedlak J; Pleskova I; Munozova H; Buc M
CORPORATE SOURCE: Cancer Research Institute, Slovak Academy of Sciences, Bratislava, Czechoslovakia.
SOURCE: NEOPLASMA, (1987) 34 (4) 417-25.
Journal code: NVO; 0377266. ISSN: 0028-2685.
PUB. COUNTRY: Czechoslovakia
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198710
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19900305
Entered Medline: 19871027

AB Monoclonal antibodies directed to MHC class II antigen(s), elicited by a non-T, non-B ALL cell line, were characterized by immunofluorescence flow cytofluorometry and ELISA immunofiltration measurements of their immunoreactivity with selected neoplastic hemopoietic cell lines, determination of their complement-dependent cytotoxic activity against isolated peripheral blood B and T lymphocytes and by two-dimensional electrophoretic analysis (isoelectric focusing, SDS-PAGE) of radiolabeled, immunoprecipitated by these antibodies cell surface antigens. Patterns of these immunological reactivities, as well as two-dimensional radioimmunoprecipitation patterns (acidic heavy chain p35 and basic light chain p30) of antigens recognized by these antibodies confirm their anti-MHC class II specificity. One of these antibodies (braFB6; IgG2b) displayed identical pattern of expression on cell lines and cell types as the typical anti-MHC **class** II antibodies, but **immunoprecipitated only a single chain** p30 radioiodinated cell surface protein (with two-dimensional pattern close to the beta-chain of MHC class II DR antigen). These properties indicate the ability of braFB6 monoclonal antibody to recognize a nonpolymorphic determinant of DP-MHC class II antigen.

L7 ANSWER 15 OF 28 MEDLINE
ACCESSION NUMBER: 83163003 MEDLINE
DOCUMENT NUMBER: 83163003 PubMed ID: 6187884
TITLE: In vitro correlate for a clonal deletion mechanism of immune response gene-controlled nonresponsiveness.
AUTHOR: Ishii N; Nagy Z A; Klein J
SOURCE: JOURNAL OF EXPERIMENTAL MEDICINE, (1983 Mar 1) 157 (3) 998-1005.
Journal code: I2V; 2985109R. ISSN: 0022-1007.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 198305
ENTRY DATE: Entered STN: 19900318
Last Updated on STN: 19900318
Entered Medline: 19830505

AB We used T cell-antigen-presenting cell (APC) combinations from two pairs of recombinant mouse strains, B10.A(4R)-B10.A(2R) and B10.S(7R)-B10.S(9R) (abbreviated 4R, 2R, 7R, 9R, respectively), which differ from each other only in the nonexpression vs. expression of cell-surface E molecules, to study the mechanism of the Ir gene-controlled (E-restricted) response to the terpolymer poly(glu51lys34tyr15) (GLT). No response to GLT occurred when the APC were from E-nonexpressor strains 4R and 7R. When APC from E-expressor strains were used and alloreactivity against the incompatible E molecules was removed by BUdR + light treatment, 7R T cells responded to GLT presented by 9R APC, but 4R T cells failed to respond to GLT presented by 2R APC. However, 4R T cells mounted a proliferative response to GLT presented by fully allogeneic 5R or 9R APC. The latter response was completely abolished by the depletion of cells alloreactive against 2R and 5R or 2R and 9R. Since removal of alloreactivity against 5R plus 9R did not affect the response of 4R T cells to GLT presented by either 5R or 9R cells, we conclude that the 4R T cells generated in response to GLT cross-react with the additional incompatibility presented by 2R cells, that is, the Ek beta chain. In contrast, 7R T cells recognizing GLT presented by 9R APC do not cross-react with Ek beta. These results demonstrate that "blind spots" in the T cell repertoire produced by depletion of cells alloreactive against a **single chain** of a **class II** MHC molecule can render a strain nonresponsive to a synthetic polypeptide antigen, and that this nonresponsiveness corresponds to that attributed to the MHC-linked Ir genes.

L7 ANSWER 16 OF 28 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:743511 CAPLUS
DOCUMENT NUMBER: 135:41597
TITLE: Single-strand conformational polymorphism and sequence polymorphism of Mhc-DRB in Latxa and Karrantzar sheep: Implications for Caprinae phylogeny
AUTHOR(S): Jugo, Begona M.; Vicario, Alberto
CORPORATE SOURCE: Animal Biology and Genetics Department, Faculty of Sciences, University of the Basque Country, Bilbao, 48080, Spain
SOURCE: Immunogenetics (2000), 51(11), 887-897
CODEN: IMNGBK; ISSN: 0093-7711
PUBLISHER: Springer-Verlag
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Single-strand conformational polymorphism anal. and DNA sequencing were used to characterize Mhc-DRB second exon variability in the Latxa and Karrantzar breeds of sheep. The presence of more than two sequences in some animals indicates that alleles of two different loci have been amplified. Six new alleles were identified by sequencing. The allele frequency distribution of the DRB1 gene is striking, with two alleles accounting for half of the gene pool in both breeds under study. The most frequent allele in both breeds was the same (named DRB1*0702), with some specific amino acids: Tyr in position 31 and Thr in 51. A species variability anal. was also performed including the entire set of sheep DRB exon 2 sequences. Based on the patchwork patterns of different alleles, interallelic recombination appears to be playing a significant role in the generation of allelic diversity at this locus in sheep. The phylogenetic tree of all known Caprinae DRB sequences shows that certain alleles from one species are more closely related to those from other species than they are to each other. Allele DRB1*0702 merits special attention due to its high similarity to the Mufflon allele. As this is the most frequent in both breeds analyzed, one can hypothesize that in sheep, both Mufflon and Argali have had different influences depending on the sheep breed under study and that the relationship between domestic sheep and Mufflon is greater than previously thought. The data generated in this study can serve as a basis for developing a typing assay for the sheep DRB genes in the Latxa and Karrantzar populations.
REFERENCE COUNT: 56 THERE ARE 56 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 17 OF 28 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:277727 CAPLUS
DOCUMENT NUMBER: 132:318607
TITLE: Sequences of a novel transcription factor of MHC class II genes, substances capable of inhibiting this new transcription factor, and medical uses of said substances
INVENTOR(S): Masternak, Krzysztof; Reith, Walter; Mach, Bernard
PATENT ASSIGNEE(S): Novimmune S.A., Switz.
SOURCE: Eur. Pat. Appl., 48 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 995798	A1	20000426	EP 1998-120085	19981024
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
WO 2000024766	A2	20000504	WO 1999-EP8026	19991022
WO 2000024766	A3	20000817		
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1124953	A2	20010822	EP 1999-970995	19991022
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				

PRIORITY APPLN. INFO.: EP 1998-120085 A 19981024
WO 1999-EP8026 W 19991022

AB The invention provides sequences of a novel transcription factor of MHC class II genes, inhibitors of this transcription factor capable of down-regulating the expression of MHC class II mols., and medical uses of these inhibitors. The novel transcription factor, called RFX-ANK, is a 33

kDa subunit of the RFX transcription complex, possesses a series of ankyrin repeats and a well defined protein-protein interaction motif, and is essential for binding the RFX complex to the conserved X box motif of MHC II promoters. The gene encoding RFX-ANK, which was mapped to 19p12, is capable of fully correcting the MHC II expression deficiency found in cell lines from patients having an autosomal recessive disease resulting from mutations in the regulatory genes responsible for the expression of MHC II genes. The invention further provides inhibitors of RFX-ANK, including antibodies, RFX-ANK mutants/derivs./fragments, ribozymes, and antisense mols. The inhibitors of the invention are also useful as immunosuppressants for the treatment and prevention of diseases assocd. with aberrant expression of MHC class II genes.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 18 OF 28 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:316302 CAPLUS
DOCUMENT NUMBER: 131:115037
TITLE: SEA-scFv as a Bifunctional Antibody: Construction of a Bacterial Expression System and Its Functional Analysis. [Erratum to document cited in CA131:57542]
AUTHOR(S): Sakurai, Naoki; Kudo, Toshio; Suzuki, Masanori; Tsumoto, Kouhei; Takemura, Shin-ichi; Kodama, Hideaki; Ebara, Shinji; Teramae, Atsushi; Katayose, Yu; Shinoda, Masao; Kurokawa, Tadashi; Hinoda, Yuji; Imai, Kohzoh; Matsuno, Seiki; Kumagai, Izumi
CORPORATE SOURCE: Tohoku Univ. School Medicine, First Department Surgery, Tohoku Univ., Sendai, Japan
SOURCE: Biochemical and Biophysical Research Communications (1999), 259(1), 230
CODEN: BBRCA9; ISSN: 0006-291X
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB On page 223, in the second and third lines of the affiliation, the dagger and double dagger symbols were reversed; the author lines were correct as printed. (c) 1999 Academic Press.

L7 ANSWER 19 OF 28 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:297317 CAPLUS
DOCUMENT NUMBER: 130:295539
TITLE: Construction of chimeric soluble MHC complexes
INVENTOR(S): Rhode, Peter R.; Acevedo, Jorge; Burkhardt, Martin; Jiao, Jin-an; Wong, Hing C.
PATENT ASSIGNEE(S): Sunol Molecular Corporation, USA
SOURCE: PCT Int. Appl., 148 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9921572	A1	19990506	WO 1998-US21520	19981013
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6232445	B1	20010515	US 1997-960190	19971029
CA 2307178	AA	19990506	CA 1998-2307178	19981013
AU 9898001	A1	19990517	AU 1998-98001	19981013
EP 1027066	A1	20000816	EP 1998-952256	19981013
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2002508300	T2	20020319	JP 2000-517730	19981013

PRIORITY APPLN. INFO.: US 1997-960190 A 19971029
WO 1998-US21520 W 19981013

AB The authors disclose the construction and expression of sol. **single-chain** (s.c.) MHC **class II** mols. In one aspect, the s.c.-MHC class II mols. include a .beta.2 chain modification, e.g., deletion of essentially the entire class II .beta.2 domain. In another aspect, the invention features **single-chain** MHC **class II** which contain an Ig light chain const. region fragment (CL). The CL fragment allows multimerization of single-chain monomers of identical or disparate MHC specificity or formation of heteromeric mols. with effector function (e.g., single-chain antibodies). In addn., the sol. MHC class II mols. can be constructed for exogenous loading of cognate peptides or the requisite peptides can be included in the single-chain constructs themselves. In one example, single-chain I-Ad mols. were constructed as fusion proteins with T-cell epitopes from either ovalbumin or glycoprotein D of herpes simplex virus. These constructs were shown to stimulate interleukin-2 prodn. by their resp. antigen-specific T-cells. MHC complexes of the invention are useful for a variety of applications including: (1) in vitro screens for identification and isolation of peptides that modulate activity of selected T-cells, including peptides that are T cell receptor antagonists and partial agonists, and (2) methods for suppressing or inducing an immune response in a mammal.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 20 OF 28 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:216936 CAPLUS
DOCUMENT NUMBER: 130:236461
TITLE: Recombinant MHC molecules useful for manipulation of antigen-specific T-cells
INVENTOR(S): Burrows, Gregory G.; Vandenbark, Arthur A.
PATENT ASSIGNEE(S): USA
SOURCE: PCT Int. Appl., 73 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9914236	A1	19990325	WO 1998-US18244	19980915

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 9893750	A1	19990405	AU 1998-93750	19980915
AU 741130	B2	20011122		
EP 1017721	A1	20000712	EP 1998-946814	19980915
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
US 6270772	B1	20010807	US 1998-153586	19980915
JP 2001516571	T2	20011002	JP 2000-511784	19980915

PRIORITY APPLN. INFO.: US 1997-64552P P 19970916
US 1997-64555P P 19971010
WO 1998-US18244 W 19980915

AB The authors disclose the prepn. and in vivo biol. activity of single-chain constructs of MHC mols. MHC class II-based mols. are comprised of covalently linked .beta.1 and .alpha.1 domains, and MHC class I-based mols. are comprised of covalently linked .alpha.1 and .alpha.2 domains. The disclosed polypeptides can be used to target antigen-specific T-cells, to detect, purify or anergize antigen-specific T-cells, and to treat conditions mediated by antigen-specific T-cells. In one example, encephalomyelitis, induced in female Lewis rats by immunization with the guinea pig myelin basic protein immunodominant epitope, was ameliorated by administration of RT1B .beta.1.alpha.1/MBP-69-89.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 21 OF 28 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:144731 CAPLUS
DOCUMENT NUMBER: 131:57542
TITLE: SEA-scFv as a Bifunctional Antibody: Construction of a Bacterial Expression System and Its Functional Analysis
AUTHOR(S): Sakurai, Naoki; Kudo, Toshio; Suzuki, Masanori; Tsumoto, Kouhei; Takemura, Shin-ichi; Kodama, Hideaki; Ebara, Shinji; Teramae, Atsushi; Katayose, Yu; Shinoda, Masao; Kurokawa, Tadashi; Hinoda, Yuji; Imai, Kohzoh; Matsuno, Seiki; Kumagai, Izumi
CORPORATE SOURCE: Tohoku University Sch. Med., First Department of Surgery, Tohoku University, Sendai, Japan
SOURCE: Biochemical and Biophysical Research Communications (1999), 256(1), 223-230
CODEN: BBRCA9; ISSN: 0006-291X
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A SEA-antibody single chain Fv (SEA-scFv) fusion protein was produced by bacterial expression system in this study. SEA-scFv has both staphylococcal enterotoxin A (SEA) effects and antibody activity directed at the epithelial mucin core protein MUC1, a cancer assocd. antigen. It was expressed mostly in the cytoplasm as an insol. form. The gene product was solubilized by guanidine hydrochloride, refolded by conventional diln. method, and purified using metal-chelating chromatog. The resulting SEA-scFv fusion protein prepn. was found to react with MUC1 and MHC class II antigens and had the ability to enhance cytotoxicity of lymphokine activated killer cells with a T cell phenotype against a human bile duct carcinoma cell line, TFK-1, expressing MUC1. This genetically engineered SEA-scFv fusion protein promises to be an important reagent for cancer immunotherapy. (c) 1999 Academic Press.

REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 22 OF 28 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:789174 CAPLUS
DOCUMENT NUMBER: 130:24116
TITLE: Production of non-immunogenic proteins by removal of T-cell and B-cell epitopes
INVENTOR(S): Carr, Francis Joseph
PATENT ASSIGNEE(S): Biovation Ltd., UK
SOURCE: PCT Int. Appl., 77 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9852976	A1	19981126	WO 1998-GB1473	19980521
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9875393	A1	19981211	AU 1998-75393	19980521
AU 736549	B2	20010802		
GB 2339430	A1	20000126	GB 1999-25632	19980521
EP 983303	A1	20000308	EP 1998-922932	19980521
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2002512624	T2	20020423	JP 1998-550129	19980521

PRIORITY APPLN. INFO.: GB 1997-10480 A 19970521
GB 1997-16197 A 19970731
GB 1997-25270 A 19971128
US 1997-67235P P 19971202
GB 1998-7751 A 19980414
WO 1998-GB1473 W 19980521

AB Proteins, or parts of proteins, may be rendered non-immunogenic, or less immunogenic, to a given species by identifying in their amino acid sequences one or more potential epitopes for T-cells of that species. Once identified, these amino acid sequence are modified to eliminate one or more MHC class II-restricted T-cell epitopes. In similar fashion, B-cell epitopes may be removed if desirable. By this process the immunogenicity of the protein when exposed to the immune system of the given species is reduced or eliminated. Monoclonal antibodies and other Ig-like mols. can particularly benefit from being rendered less

immunogenic (e.g., humanized antibodies for therapy).
REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 23 OF 28 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:734956 CAPLUS
DOCUMENT NUMBER: 129:314972
TITLE: Enhancing the binding affinity of peptides for MHC class II molecules.
INVENTOR(S): Nag, Bishwajit
PATENT ASSIGNEE(S): Anergen Inc., USA
SOURCE: U.S., 24 pp. Cont.-in-part of U.S. Ser. No. 227,372.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5824315	A	19981020	US 1996-640344	19960430
US 5763585	A	19980609	US 1994-227372	19940414
US 6090587	A	20000718	US 1995-470535	19950606
EP 973547	A1	20000126	EP 1997-919885	19970318
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRIORITY APPLN. INFO.:
US 1993-143575 B2 19931025
US 1994-227372 A2 19940414
US 1994-329010 A2 19941025
US 1993-136216 B2 19931013
US 1996-640344 A 19960430
WO 1997-US4360 W 19970318

AB This invention provides methods of improving the binding affinity of a peptide epitope for MHC class II mols. by attaching to the N-terminus of the peptide epitope a hydrophobic amino acid or a peptide contg. a hydrophobic amino acid. In one example, a peptide fragment of myelin basic protein, modified with an N-terminal tyrosine, exhibits enhanced binding to HLA-DR2. The invention also describes complexes between the modified antigenic peptides and MHC **class II** mols. (as **single-chain** constructs or fusion proteins) and their potential application in autoimmune disorders.

L7 ANSWER 24 OF 28 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:533677 CAPLUS
DOCUMENT NUMBER: 127:204455
TITLE: Preparation and immunomodulatory activity of single-chain MHC mols.
INVENTOR(S): Rhode, Peter R.; Jiao, Jin-An; Burkhardt, Martin; Wong, Hing C.
PATENT ASSIGNEE(S): Dade International, Inc., USA; Rhode, Peter R.; Jiao, Jin-An; Burkhardt, Martin; Wong, Hing C.
SOURCE: PCT Int. Appl., 216 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9728191	A1	19970807	WO 1997-US1617	19970130
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
US 5869270	A	19990209	US 1996-596387	19960131
CA 2244755	AA	19970807	CA 1997-2244755	19970130
AU 9722538	A1	19970822	AU 1997-22538	19970130
AU 729672	B2	20010208		
EP 877760	A1	19981118	EP 1997-905709	19970130
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2000515363	T2	20001121	JP 1997-527863	19970130
US 6309645	B1	20011030	US 1998-67615	19980428
US 2002034513	A1	20020321	US 2001-848164	20010503

PRIORITY APPLN. INFO.:
US 1996-596387 A 19960131
WO 1997-US1617 W 19970130
US 1998-67615 XX 19980428

AB The present invention relates to novel complexes of major histocompatibility complex (MHC) mols. and uses of such complexes. In one aspect, the invention relates to loaded MHC complexes that include at least one MHC mol. with a peptide-binding groove and a presenting peptide non-covalently linked to the MHC protein. In another aspect, the invention features **single chain** MHC **class II** peptide fusion complexes with a presenting peptide covalently linked to the peptide binding groove of the complex. MHC complexes are useful for a variety of applications including: (1) in vitro screens for identification and isolation of peptides that modulate activity of selected T cells, including peptides that are T cell receptor antagonists and partial agonists, and (2) methods for suppressing or inducing an immune response in a mammal.

L7 ANSWER 25 OF 28 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:458043 CAPLUS
DOCUMENT NUMBER: 125:112746
TITLE: Single chain T-cell receptor
INVENTOR(S): Strominger, Jack L.; Chung, Shan
PATENT ASSIGNEE(S): President and Fellows of Harvard College, USA
SOURCE: PCT Int. Appl., 49 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9618105	A1	19960613	WO 1995-US15696	19951204
W: CA, JP				

RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
PRIORITY APPLN. INFO.: US 1994-349915 19941206
AB Disclosed is a single chain T-cell receptor which binds specifically to an MHC peptide ligand. The single chain T-cell receptor is a 3-domain construct comprising an .alpha. chain variable domain, a .beta. chain variable domain and a const. domain. Also disclosed is a method using the self-signaling single chain T-cell receptor, which binds specifically to an MHC/peptide ligand wherein the peptide component is a viral specific T-cell epitope, and antigen-presenting cells contg. the MHC/peptide complex on their surface and measuring the binding for diagnosing viral infection.

L7 ANSWER 26 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:6971 BIOSIS
DOCUMENT NUMBER: PREV200200006971
TITLE: MHC molecules and uses thereof.
AUTHOR(S): Rhode, Peter R.; Jiao, Jin-An (1); Burkhardt, Martin; Wong, Hing C.
CORPORATE SOURCE: (1) Fort Lauderdale, FL USA
ASSIGNEE: Sunol Molecular Corporation
PATENT INFORMATION: US 6309645 October 30, 2001
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Oct. 30, 2001) Vol. 1251, No. 5, pp. No Pagination. e-file.
ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
AB The present invention relates to novel complexes of major histocompability complex (MHC) molecules and uses of such complexes. In one aspect, the invention relates to loaded MHC complexes that include at least one MHC molecule with a peptide-binding groove and a presenting peptide non-covalently linked to the MHC protein. In another aspect, the invention features **single chain** MHC **class II** peptide fusion complexes with a presenting peptide covalently linked to the peptide binding grove of the complex. MHC complexes of the invention are useful for a variety of applications including: 1) in vitro screens for identification and isolation of peptides that modulate activity of selected T cells, including peptides that are T cell receptor antagonists and partial agonists, and 2) methods for suppressing or inducing an immune response in a mammal.

L7 ANSWER 27 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:499745 BIOSIS
DOCUMENT NUMBER: PREV200100499745
TITLE: Soluble MHC complexes and methods of use thereof.
AUTHOR(S): Rhode, Peter R.; Acevedo, Jorge (1); Burkhardt, Martin; Jiao, Jin-an; Wong, Hing C.
CORPORATE SOURCE: (1) Miami, FL USA
ASSIGNEE: Sunol Molecular Corporation
PATENT INFORMATION: US 6232445 May 15, 2001
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (May 15, 2001) Vol. 1246, No. 3, pp. No Pagination. e-file.
ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
AB The present invention relates to novel complexes of major histocompability complex (MHC) molecules and uses of such complexes. In one aspect, the invention relates to **single chain** MHC **class II** complexes that include a **class II** beta2 chain modification, e.g., deletion of essentially the entire **class II** beta2 chain. In another aspect, the invention features **single chain** MHC **class II** which comprise an immunoglobin constant chain or fragment. Further provided are polyspecific MHC complexes comprising at least one **single chain** MHC **class II** molecule. MHC complexes of the invention are useful for a variety of applications including: 1) in vitro screens for identification and isolation of peptides that modulate activity of selected T cells, including peptides that are T cell receptor antagonists and partial agonists, and 2) methods for suppressing or inducing an immune response in a mammal.

L7 ANSWER 28 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:246173 BIOSIS
DOCUMENT NUMBER: PREV199900246173
TITLE: Single chain MHC complexes and uses thereof.
AUTHOR(S): Rhode, P. R.; Jiao, J-A.; Burkhardt, M.; Wong, H. C.
CORPORATE SOURCE: Miami, Fla. USA
ASSIGNEE: SUNOL MOLECULAR CORPORATION
PATENT INFORMATION: US 5869270 Feb. 9, 1999
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Feb. 9, 1999) Vol. 1219, No. 2, pp. 1524.
ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English

=> dis his

(FILE 'HOME' ENTERED AT 15:26:37 ON 04 MAY 2002)

FILE 'MEDLINE, CAPLUS, EMBASE, BIOSIS' ENTERED AT 15:26:47 ON 04 MAY 2002
L1 4867 S RHODE P?/AU OR JIAO J?/AU OR BURKHARDT M?/AU OR WONG H?/AU
L2 16 S L1 AND MHC
L3 12 DUP REM L2 (4 DUPLICATES REMOVED)
L4 62 S (SINGLE (1N) CHAIN) (10N) (CLASS (1N) II)
L5 30 DUP REM L4 (32 DUPLICATES REMOVED)
L6 2 S L5 AND PD<19960131
L7 28 S L5 NOT L6

=> s tetramer and MHC
AND IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s tetramer (P) MHC
L8 766 TETRAMER (P) MHC

=> s l8 and PD<19960131
'19960131' NOT A VALID FIELD CODE
3 FILES SEARCHED...
L9 23 L8 AND PD<19960131

=> dup rem l9
PROCESSING COMPLETED FOR L9
L10 11 DUP REM L9 (12 DUPLICATES REMOVED)

=> dis l10 1-11 ibib abs

L10 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 1
ACCESSION NUMBER: 1996:608417 CAPLUS
DOCUMENT NUMBER: 125:245038
TITLE: Phenotypic analysis of antigen-specific T lymphocytes
AUTHOR(S): Altman, John D.; Moss, Paul A. H.; Goulder, Philip J. R.; Barouch, Dan H.; McHeyzer-Williams, Michael G.; Bell, John I.; McMichael, Andrew J.; Davis, Mark M.
CORPORATE SOURCE: Sch. Medicine, Stanford Univ., Stanford, CA, 94305-5428, USA
SOURCE: Science (Washington, D. C.) (1996), 274(5284), 94-96
CODEN: SCIEAS; ISSN: 0036-8075
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Identification and characterization of antigen-specific T lymphocytes during the course of an immune response is tedious and indirect. To address this problem, the peptide-major histocompatibility complex (MHC) ligand for a given population of T cells was multimerized to make sol. peptide-MHC **tetramers**. **Tetramers** of human lymphocyte antigen A2 that were complexes with two different human immunodeficiency virus (HIV)-derived peptides or with a peptide derived from influenza A matrix protein bound to peptide-specific cytotoxic T cells in vitro and to T cells from the blood of HIV-infected individuals. In general, **tetramer** binding correlated well with cytotoxicity assays. This approach should be useful in the anal. of T cells specific for infectious agents, tumors, and autoantigens.

Oct 4, 1996

L10 ANSWER 2 OF 11 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1996:509704 BIOSIS
DOCUMENT NUMBER: PREV199699232060
TITLE: Phenotypic analysis of antigen-specific T lymphocytes.
AUTHOR(S): Altman, John D.; Moss, Paul A. H.; Goulder, Philip J. R.; Barouch, Dan H.; McHeyzer-Williams, Michael G.; Bell, John I.; McMichael, Andrew J.; Davis, Mark M. (1)
CORPORATE SOURCE: (1) Howard Hughes Med. Inst., Dep. Microbiol. Immunol., Beckman Center, Room B221, Stanford Univ., Stanford, CA 94305-5428 USA
SOURCE: Science (Washington D C), (1996) Vol. 275, No. 5284, pp. 94-96.
ISSN: 0036-8075.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Identification and characterization of antigen-specific T lymphocytes during the course of an immune response is tedious and indirect. To address this problem, the peptide-major histocompatability complex (MHC) ligand for a given population of T cells was multimerized to make soluble peptide-MHC **tetramers**. **Tetramers** of human lymphocyte antigen A2 that were complexed with two different human immunodeficiency virus (HIV)-derived peptides or with a peptide derived from influenza A matrix protein bound to peptide-specific cytotoxic T cells in vitro and to T cells from the blood of HIV-infected individuals. In general, **tetramer** binding correlated well with cytotoxicity assays. This approach should be useful in the analysis of T cells specific for infectious agents, tumors, and autoantigens.

L10 ANSWER 3 OF 11 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:389271 CAPLUS
DOCUMENT NUMBER: 125:83729
TITLE: Enumeration and characterization of memory cells in the TH compartment
AUTHOR(S): McHeyzer-Williams, Michael G.; Altman, John D.; Davis, Mark M.
CORPORATE SOURCE: Medical Center, Duke University, Durham, NC, 27710, USA
SOURCE: Immunol. Rev. (1996), 150, 5-21
CODEN: IMRED2; ISSN: 0105-2896
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with 44 refs. Discussed are: lymphocyte differentiation and repertoire maturation in vivo; the H-2k-restricted pigeon cytochrome C (PCC)-specific response; emergence of a PCC-specific helper T-cell response in TCR transgenic mice; primary and memory PCC-specific helper T-cell response in normal mice; repertoire selection and clonal maturation in the helper T-cell compartment; 5-color flow cytometry for anal. of the developing immune response in vivo; and direct labeling of specific T-cells using peptide/MHC **tetramers**.

L10 ANSWER 4 OF 11 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 95131713 EMBASE
DOCUMENT NUMBER: 1995131713
TITLE: Hotspots of homologous recombination in mouse meiosis.
AUTHOR: Shiroishi T.; Koide T.; Yoshino M.; Sagai T.; Moriwaki K.
CORPORATE SOURCE: Mammalian Genetics Laboratory, National Institute of Genetics,Mishima, Shizuoka 411, Japan
SOURCE: Advances in Biophysics, (1995) 31/- (119-132).
ISSN: 0065-227X CODEN: ADVBAT
COUNTRY: Japan
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The molecular mapping of recombinational breakpoints in the proximal region of the mouse MHC has revealed four hotspots at which breakpoints are clustered. A direct comparison of the nucleotide sequences of two independent hotspots revealed common molecular elements: a consensus sequence of the middle-repetitive MT-family, a repeat of **tetramer** sequences and a sequence homologous to a solitary LTR of mouse retroviruses. Extremely high frequency of recombination is observed at these hotspots when particular MHC haplotypes are used in genetic crosses. Wild mouse-derived wm7 haplotype instigates recombination at the hotspot located at the 3'-end of the Lmp-2 gene only during female meiosis. Pine genetic analysis demonstrated that the wm7 haplotype carries a genetic factor to instigate recombination and another factor to suppress recombination specifically during male meiosis. In addition, there is no dose effect of the hotspot on frequency of recombination. Finally, we described an attempt to establish an efficient in vitro assay system for

monitoring recombination using plasmid DNAs that contain the Lmp-2 hotspot and nuclear extracts prepared from mouse testis.

L10 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 2
ACCESSION NUMBER: 1994:577167 CAPLUS
DOCUMENT NUMBER: 121:177167
TITLE: An LI and ML motif in the cytoplasmic tail of the MHC-associated invariant chain mediate rapid internalization
AUTHOR(S): Bremnes, Bjorn; Madsen, Toril; Gedde-Dahl, Merete; Bakke, Oddmund
CORPORATE SOURCE: Department Biology, University Oslo, Oslo, N-0316, Norway
SOURCE: J. Cell Sci. (1994), 107(7), 2021-32
CODEN: JNCSAI; ISSN: 0021-9533
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Invariant chain (Ii) is a transmembrane protein that assocs. with the MHC class II mols. in the endoplasmic reticulum. Two regions of the 30 residue cytoplasmic tail of Ii contain sorting information able to direct Ii to the endocytic pathway. The full-length cytoplasmic tail of Ii and the two tail regions were fused to neuraminidase (NA) forming chimeric proteins (INA). Ii is known to form trimers and when INA was transfected into COS cells it assembled as a **tetramer** like NA. The INA mols. were targeted to the endosomal pathway and contransfection with Ii showed that both mols. appeared in the same vesicles. By labeling the INA fusion proteins with iodinated antibody it was found that mols. with either endocytosis signal were expressed at the plasma membrane and internalized rapidly. Point mutations revealed that an LI motif within the first region of the cytoplasmic tail and an ML motif in the second region were essential for efficient internalization. The region contg. the LI motif is required for Ii to induce large endosomes, but a functional LI internalization motif was not fundamental for this property. The cytoplasmic tail of Ii is essential for efficient targeting of the class II mols. to endosomes and the dual LI and ML motif may thus be responsible for directing these mols. to the endosomal pathway, possibly via the plasma membrane.

L10 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 3
ACCESSION NUMBER: 1994:653228 CAPLUS
DOCUMENT NUMBER: 121:253228
TITLE: Immunological significance of invariant chain from the aspect of its structural homology with the cystatin family
AUTHOR(S): Katunuma, Nobuhiko; Kakegawa, Hisao; Matsunaga, Youichi; Saibara, Toshiji
CORPORATE SOURCE: Inst. Health Sci., Tokushima Bunri Univ., Tokushima, 770, Japan
SOURCE: FEBS Lett. (1994), 349(2), 265-9
CODEN: FEBLAL; ISSN: 0014-5793
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The primary structure of p31 of invariant chain (Ii-chain) shows about 50% homol. with those of the cystatin family which are endogenous cysteine protease inhibitors. The binding domains between Ii-chain and HLA-DR7 were estd. from the structural homol. between cystatin and Ii-chain and also between cathepsins and DR7, resp. The QL64-71 and GS76-88 of Ii-chain were estd. to be the binding domains with GG45-51 and VS57-63 of HLA-DR7, resp. The purified human Ii-chain from spleen is capable of forming 4 mol. forms from monomer to **tetramer** by redox-potential dependent disulfide bond formation. The Ii-chain inhibits cathepsin L and H competitively as a dimer and the Ki value for cathepsin L was 4.1.times.10-8 M, but cathepsin B was not inhibited at all. The Ii-chain showed mainly a dimer (60 kDa) under the assay condition of cathepsins with cysteine and was not degraded by these cathepsins. The Ii-chain may play an important role in the regulation of antigenic peptide presentation to MHC class II.

L10 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 4
ACCESSION NUMBER: 1992:192281 CAPLUS
DOCUMENT NUMBER: 116:192281
TITLE: Role of the transmembrane and cytoplasmic domains of surface IgM in endocytosis and signal transduction
AUTHOR(S): Dubois, Patrice M.; Stepinski, Jan; Urbain, Jacques; Sibley, Carol Hopkins
CORPORATE SOURCE: Dep. Genet., Univ. Washington, Seattle, WA, 98195, USA
SOURCE: Eur. J. Immunol. (1992), 22(3), 851-7
CODEN: EJIMAF; ISSN: 0014-2980
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The crosslinking of membrane IgM (mIgM) triggers the activation and differentiation of B lymphocytes. One very rapid result of the crosslinking is the activation of phospholipase C, the subsequent mobilization of free Ca from internal stores and the activation of protein kinase C. This is followed by a redistribution of the receptor-ligand complexes to a small cap on the B cell surface, the first step in endocytosis and antigen processing. Crosslinking of major histocompatibility complex (MHC) class I neither stimulates the release of intracellular Ca nor does it induce capping and endocytosis of the cell surface receptors. In this study, the role was detd. of 2 C-terminal domains of the .mu. heavy chain in signal transduction, capping, and endocytosis of mIgM. Advantage was taken of the clear differences between MHC class I mols. and mIgM, replacing the transmembrane and cytoplasmic domains of .mu. by their MHC class I equiv. The results show that the hybrid heavy chain could still assoc. with light chains and assemble into a **tetramer** on the cell surface. However, crosslinking of the hybrid cell receptor produced neither release of Ca from internal stores, nor capping and endocytosis. Thus, the 2 C-terminal domains of .mu. are crit. to both signal transduction and modulation of the mIgM-ligand complexes from the surface of B lymphocytes.

L10 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 5
ACCESSION NUMBER: 1992:253705 CAPLUS
DOCUMENT NUMBER: 116:253705
TITLE: Tetrameric cell-surface MHC class I molecules
AUTHOR(S): Krishna, Sudhir; Benaroch, Philippe; Pillai, Shiv
CORPORATE SOURCE: Massachusetts Gen. Hosp., Harvard Med. Sch., Boston, MA, 02129, USA
SOURCE: Nature (London) (1992), 357(6374), 164-7
CODEN: NATUAS; ISSN: 0028-0836
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Purified major histocompatibility complex (MHC) class I mols. have been studied at high resoln. by x-ray crystallog.; the structure is a complex of a single heavy chain, a .beta.2-microglobulin light chain and a tightly bound peptide moiety. Complete MHC class I mols. are postranslationally assembled into **tetramers** (made up of 4 heavy chains and 4 .beta.2-microglobulin units), and this tetrameric species is expressed on the cell surface. The multivalent tetrameric structure of class I mols. can be reconciled with models of T-cell activation that invoke antigen-receptor crosslinking, as opposed to models that depend on an allosteric change.

L10 ANSWER 9 OF 11 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1992:525666 CAPLUS
DOCUMENT NUMBER: 117:125666
TITLE: Diversity and evolution at the Eb recombinational hotspot in the mouse
AUTHOR(S): Sant'Angelo, D.; Heine, D.; Passmore, H.
CORPORATE SOURCE: Bur. Biol. Res., Rutgers Univ., Piscataway, NJ, 08855, USA
SOURCE: NATO ASI Ser., Ser. H (1991), 59(Mol. Evol. Major Histocompat. Complex), 473-82
CODEN: NASBE4; ISSN: 1010-8793
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The 2nd intron of the mouse Eb gene contains a well-defined recombinational hotspot. A comparison of std. lab. MHC haplotypes suggests a region of 650 bp in the 3' end of this intron, corresponding to the location of the breakpoint region of the recombinational hotspot, is highly conserved. This conserved segment was examd. in several species and subspecies of Mus and revealed nucleotide diversity ranging 0-4.3%. Sequence anal. also resulted in the identification of a variable mononucleotide repeat within the proposed recombinational hotspot which shows .gtoreq.8 different sequence configurations. In addn., a variable no. tandem repeat (VNTR), previously identified in the 3' end of the intron, was examd. for diversity. This anal. revealed a wide variation in the no. of AGGC repeats obsd. ranging from as few as 5 (M. caroli) to as many as 21 (M. musculus brevirostris). Further, a 2nd VNTR, consisting of the **tetramer** TGGA was also detected immediately preceding the AGGC tandem repeat.

L10 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 6
ACCESSION NUMBER: 1989:476040 CAPLUS
DOCUMENT NUMBER: 111:76040
TITLE: Class II restriction in mice to the malaria candidate vaccine ring infected erythrocyte surface antigen (RESA) as synthetic peptides or as expressed in recombinant vaccinia
AUTHOR(S): Lew, Andrew M.; Langford, Christopher J.; Pye, David; Edwards, Stirling; Corcoran, Lyn; Anders, Robin F.
CORPORATE SOURCE: Walter and Eliza Hall Inst. Med. Res., R. Melbourne Hosp., Melbourne, 3050, Australia
SOURCE: J. Immunol. (1989), 142(11), 4012-16
CODEN: JOIMA3; ISSN: 0022-1767
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The immune response to 3 peptides corresponding to the repeat regions of the malaria candidate vaccine ring infected E surface antigen (RESA) were studied. Both antibody responses and lymphocyte stimulation in mice injected with these peptides without carrier were restricted to certain MHC class II haplotypes. Mice bearing IAk were strong responders to all 3 peptides. Mice bearing IAd were strong responders only to the 3' repeat peptides, the octamer and **tetramer**. Mice bearing Isor Iq did not respond to any repeat peptides. Remarkably, the pattern of genetic restriction of the antibody response to the entire RESA as expressed in vaccinia indicated that there were no other epitopes besides the 3 repeats. Because only one class II haplotype (i.e., k) out of 5 responded strongly to this peptide and only 2 out of 5 (i.e., k and d) responded to the octamer or **tetramer**, it may be difficult to achieve a good immune response against RESA in most or all humans.

L10 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 7
ACCESSION NUMBER: 1986:620043 CAPLUS
DOCUMENT NUMBER: 105:220043
TITLE: Molecular analysis of the hotspot of recombination in the murine major histocompatibility complex
AUTHOR(S): Kobori, Joan A.; Strauss, Erich; Minard, Karyl; Hood, Leroy
CORPORATE SOURCE: Div. Biol., California Inst. Technol., Pasadena, CA, 91125, USA
SOURCE: Science (Washington, D. C., 1883-) (1986), 234(4773), 173-9
CODEN: SCIEAS; ISSN: 0036-8075
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Biol. and serol. assays were used to define 4 subregions for the I region of the major histocompatibility complex (MHC) in the order I-A, I-B, I-J, and I-E. The I-J subregion presumably encodes the I-J polypeptide of the elusive T-cell suppressor factors. Restriction enzyme site polymorphisms and DNA sequence analyses of the I region from 4 recombinant mouse strains were used to localize the putative I-B and I-J subregions to a 1.0-kilobase (kb) region within the E.beta. gene. Sequencing this region from E.beta. clones derived from the 2 mouse strains: B10.A(3R), I-Jb and B10.A(5R), I-Jk initially used to define the I-J subregion revealed that these regions are identical, hence the distinct I-Jb and I-Jk mols. cannot be encoded by this DNA. In addn., the DNA sequence data also refute the earlier mapping of the I-B subregion. Anal. of the DNA sequences of 3 parental and 4 I region recombinants reveals that the recombinant events in 3 of the recombinant strains occurred within a 1-kb region of DNA, supporting the proposition that a hotspot for recombination exists in the I region. The only striking feature of this hotspot is a **tetramer** repeat (AGGC)n that shows 80% homol. to the minisatellite sequence which may facilitate recombination in human chromosomes.

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FILE 'MEDLINE, CAPLUS, EMBASE, BIOSIS' ENTERED AT 15:26:47 ON 04 MAY 2002
L1 4867 S RHODE P?/AU OR JIAO J?/AU OR BURKHARDT M?/AU OR WONG H?/AU
L2 16 S L1 AND MHC
L3 12 DUP REM L2 (4 DUPLICATES REMOVED)

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L4            62 S (SINGLE (1N) CHAIN) (10N) (CLASS (1N) II)
L5            30 DUP REM L4 (32 DUPLICATES REMOVED)
L6             2 S L5 AND PD<19960131
L7            28 S L5 NOT L6
L8           766 S TETRAMER (P) MHC
L9            23 S L8 AND PD<19960131
L10           11 DUP REM L9 (12 DUPLICATES REMOVED)

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